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SPERMATOGENESIS OF THE BLACK-CLAWED CRAB, *LOPHOPANOPEUS BELLUS* (STIMPSON) RATHBUN.

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INTRODUCTION.

During the past few years I have been studying various cytological aspects of the gonads of numerous Pacific coast Brachyura (Fasten, '17, '18, '21 and '24) giving particular attention to the problem of spermatogenesis. The present paper is a continuation of the above research, dealing with the maturation of the male gametes of the black-clawed crab, *Lophopanopeus bellus* (Stimpson) Rathbun. This Brachyuran is a native of the Pacific coast of the United States, and according to Schmitt ('21), it ranges from Alaska to southern California. The material upon which the present contribution is based was obtained mainly along the shores of Puget Sound. In certain localities of this region, particularly in the San Juan islands, the black-clawed crab is found to be quite abundant.

MATERIAL AND METHODS.

The material used consisted of smear preparations of the testis of the crustacean under consideration. Although sectioned material was available, nevertheless the smears were found to be so superior that all of the stages and drawings were derived from them. The time for obtaining the best material was the latter part of June and the first portion of July. These periods agree quite well with what has been found to be the case in the testes of other Brachyura.

The smears were prepared in the usual manner, as described in my earlier paper on the spermatogenesis of the edible crab, *Cancer magister* Dana (Fasten, '18). Numerous fixatives were tried, but Flemming's strong solution was found to yield the best results. This mixture made the principal parts of the cell stand out clearly. Furthermore, it produced some differentiation in the staining capacities of the chromatoid bodies and the chromosomes. The former invariably stained less heavily than the latter. This was particularly noticeable when the slides were considerably destained. As for staining, the best results were obtained with Heidenhain's iron-alum hæmatoxylin and a counter-stain of acid-fuchsin.

The mature radial spermatozoa were studied in various isotonic, hypotonic and hypertonic solutions in order to discover the manner in which they function. These experiments have already been published in a separate paper (Fasten, 1921) and therefore the results will not be repeated here.

Description of Male Gonads.

The male reproductive organs of *Lophopanopeus bellus* lie in the cephalothoracic region. They consist of a bilobed tubular testis and a pair of vas deferent ducts. Each lobe of the testis runs laterally in the space between the heart and the digestive glands. Directly below the anterior portion of the heart the testicular lobes unite and from this junction point the convoluted vas deferent ducts originate. These run posteriorly to the base of the fifth pair of walking legs where they open to the outside.

The male gonads attain their maximum size during the latter part of June and the early portion of July. Between these times the cells in the tubules of the testis are undergoing rapid proliferation and all stages in the spermatogenesis process may be secured. Typical cross-sections of the testis reveal a similar picture to that given by me in 1918 for the edible crab, *Cancer magister* Dana. Some of the tubules contain just one typical stage; others show two distinct stages, while still others reveal three steps in the maturation process.

In the posterior region of the testis, where the lobes unite, and in the vas deferent ducts are found the mature spermatozoa. In *Lophopanopeus bellus*, the mature spermatozoa within the distal

ends of the vas deferent ducts are surrounded by a single large spermatophore. This is contrary to what has been found in the other Brachyura studied, where numerous spermatophores are developed.

SPERMATOGENESIS.

A. Spermatogonial Stages.

In the black-clawed crab two distinct spermatogonial divisions can be seen (Figs. 1–2 and 8–11). The primary spermatogonial stages (Figs. 1–2) are considerably larger than those of the secondary spermatogonia (Figs. 8–11) but otherwise the division proceeds in the same manner as seen in Figs. 8–11. The resting primary spermatogonium (Fig. 1) is almost twice the size of the resting secondary spermatogonium (Fig. 8). Their structure, however, is similar. Numerous chromatin clumps and linin strands may be distinguished within the nucleus. A large nucleolus may also be recognized. The cytoplasm is uniform throughout, with the exception that occasionally larger masses that stain somewhat like chromatin may be seen. These masses are clearly discernible in Fig. 1. The centrosome which is a single granule is nearly always present.

When any one of the spermatogonia divides, the chromatin within the nucleus begins to fragment until a great many large, heavily staining clumps appear, as shown in Fig. 2. The nuclear wall soon breaks down with the result that the cell enters the metaphase stage of division (Fig. 9). Polar views of the metaphase showed the chromosomes to be rather small and numerous. Accurate counts could not be made although in many cases over one hundred of them were found distributed throughout the equatorial plane. But since it has been definitely determined that there are 62 bivalent chromosomes in the reduced number, there must be 124 univalent ones in the spermatogonial counts.

The anaphase (Fig. 10) and telophase (Fig. 11) stages follow quickly, thereby completing the spermatogonial divisions. The division of the secondary spermatogonia produces the resting primary spermatocytes (Fig. 12) which then continue the maturation process.

In connection with the spermatogonial stages reference must be made to the so-called "nutritive cells" (Figs. 3–7) which are

commonly found associated with the spermatogonial strips located in tubules where there are mature spermatozoa. These so-called "nutritive cells" are huge structures assuming numerous shapes. Typical examples are pictured in Figs. 3–7. The delineation between the cells is oftentimes effaced, so that the nuclei seem to lie in a syncitial mass of cytoplasmic material. There is considerable difference of opinion amongst cytologists as to the probable relation between the nutritive and spermatogonial cells of decapod Crustacea. Grobben ('78), Gilson ('86) and Herrmann ('90) look upon the nutritive cells as foundation germinal cells from which the spermatogonia originate. In contrast to this view, such workers as St. George ('92), and Keppen ('06) regard the nutritive cells as being derived through a transformation of spermatogonia which have not undergone maturation. My own observations are in accord with the latter view. Such stages like Figs. 3 and 4 resemble primary spermatogonial cells (Fig. 1) but are larger in size.

Many of the workers on the cytology of the Decapoda have claimed that the nutritive cells undergo amitotic division. In sectioned material it seems as if this were the case. For instance certain sections through such cells as illustrated in Figs. 5 and 6 would yield typical examples for those advocating amitosis. However, when smears of the entire cells were obtained, the true condition is discovered. I have found numerous indications that mitosis is the normal process of division in these nutritive cells. A good review of the literature on this subject can be obtained in my earlier paper on the spermatogenesis of the American crayfish, *Cambarus virilis* (Fasten, '14).

B. Primary Spermatocyte Stages.

In the primary spermatocyte stages, the cells undergo growth, synapsis and reduction in the number of chromosomes. A single chromatoid body makes its appearance within the cytoplasm during the early stages of the growth period. The general sequence of events is quite similar to that found in other decapod Crustacea (Fasten, '14, '18, '24).

Growth Period.—The resting primary spermatocyte (Fig. 12) is slightly smaller than the resting secondary spermatogonium

(Fig. 8). Within its nucleus may be seen numerous irregular chromatin clumps distributed along a meshwork of thin strands. A single centrosome can also be distinguished.

When the primary spermatocyte begins its process of development, the first noticeable change occurs in the nucleus. The chromatin clumps fragment (Figs. 13 and 14) and soon weave out into a large number of leptotene threads (Fig. 15). These threads are distinct but so numerous that it was impossible to get any count of them.

The next step in the growth process is the pre-synaptic stage (Fig. 16) in which the leptotene threads wander to one pole of the nucleus and become lined up side by side into pairs. Within the cytoplasm a single, spherical chromatoid body makes its appearance and it is invariably surrounded by a clear area. This is quite similar to the bodies found by Wilson ('13), Komai ('20) and myself ('14, '18 and '24) for other forms. In slides which were considerably destained the chromatoid body took on a lighter hue than the chromatin of the nucleus. I found this same differentiation in destained preparations of *Cancer productus* Randall, *Cancer oregonensis* (Dana) Rathbun, and *Cancer gracilis* Dana which were fixed with Flemming's strong solution. From this stage on the chromatoid body persists and can be easily distinguished.

The pachytene stage follows (Fig. 17) in which synapsis of the paired leptotene threads takes place thereby transforming them into distinct gemini. In many of the preparations the line of fusion between some of the leptotene threads could be clearly seen. This is similar to the condition found in *Cambarus virilis* (Fasten '14), but differs from that found in the various Cancer crabs studied (Fasten, '18 and '24) where the fusion between the leptotene threads is complete.

The diplotene and post-diplotene stages (Figs. 18 and 19) follow each other in logical sequence. In the diplotene stage the paired threads of each geminus open up at one end along the longitudinal plane of fusion but remain attached at the opposite end, thereby producing figures like V, 8 and U. At the same time a second longitudinal furrow occurs along each of the opened arms, thus converting each geminus into four thin strands

attached at one end, as seen in some of the V's, U's and 8's of Fig. 18.

The four components of each geminus continue to open up in such a manner as to produce typical X's like those shown in Fig. 19. This transformation marks the end of the post-diplotene stage and the cell is now ready to terminate the growth period by the formation of tetrads.

Figure 20 shows the pre-tetrad condition. The opposite ends of the X's soon wander towards each other and the middle fusion point becomes effaced. The result is that every one of the X's is converted into four thin strands, having the appearance of a pair of parallel lines with a transverse split in the middle.

Through a further condensation, each of the four thin strands is modified into a spherical chromosome, thus converting every geminus into a tetrad (Figs. 20 and 21). Following this, the typical bivalent dumb-bells are formed through a fusion of the pairs of univalent chromosomes of the tetrads. This is particularly well shown in Fig. 21. Here, also, the centrosome has divided into two elements and each is seen to migrate away from the other. At the same time the nuclear wall has begun to disintegrate and the cell, in general, has terminated its process of growth.

A careful study of the various stages of growth shows that the chromatoid body makes its appearance in the pre-synapsis period and persists from then on. A centrosome and an idiozome are clearly visible. As for synapsis, it occurs in parasynaptic fashion in which there is a side-by-side conjugation of the chromosomes. This is similar to what happens in the other decapod Crustacea which I have studied (Fasten, '14, '18 and '24). Komai ('20) has also found parasynapsis in the crustacean *Squilla oratoria* de Haan.

Reduction Division.—Following the disintegration of the nuclear wall, the primary spermatocyte enters the metaphase period (Figs. 22 and 23). The bivalent dumb-bells are lined up in the equatorial plane, and from them the delicate spindle fibres converge towards the centrosomes which are located at opposite poles. The single chromatoid body wanders undivided towards one of the poles of the cell. In some instances it lies amongst

the spindle fibres (Fig. 22) while at other times it is located outside the spindle proper, as shown in Fig. 23. As already mentioned, when the slides were greatly destained, the chromatoid body could be easily distinguished from the chromosomes as it took on a lighter color. The most consistent counts of polar views of metaphase stages showed 62 bivalent chromosomes distributed throughout the plane of the equator (Fig. 24).

The anaphase and telophase stages (Figs. 25–28) follow quickly, ultimately producing the secondary spermatocytes. Figure 28 shows the two types of secondary spermatocytes formed, one containing the single chromatoid body, while the other is devoid of any such structure. These two kinds of secondary spermatocytes occur in equal number.

C. Secondary Spermatocyte Stages.

The secondary spermatocyte divisions are equational. No rest period is found after the mitosis of the primary spermatocyte, the cells immediately enter the metaphase to undergo rapid division. No good counts of the chromosomes of secondary spermatocytes could be made. The cells on the whole are almost half the size of the primary spermatocytes.

Figures 29–34 show the typical stages in the division of the secondary spermatocyte which possesses a chromatoid body, while Figs. 35–38 show the same process in the secondary spermatocyte that is minus the chromatoid body. The ultimate result of all these divisions is the formation of two kinds of spermatids, one which contains a chromatoid body (Fig. 39) and the other that lacks it (Fig. 44). This latter spermatid is about three times as numerous as the former one.

D. Transformations of Spermatids.

The two types of resting spermatids (Figs. 39 and 44) are spherical cells which have large masses of chromatin within the nuclei that stain intensely black with Heidenhain's hæmatoxylin. The cytoplasm is uniformly granular, possessing a distinct centrosome. The chromatoid body can be clearly seen within one of the kinds of spermatids (Fig. 39).

Transformations of the two spermatids occur in similar fashion.

The first change is a disintegration of the dense chromatin of the nucleus. This is consistently reduced until there are two spherical granules (Fig. 45) and then one (Figs. 40 and 41) left. These single granules appear like karyosomes. While all this is going on the nucleus takes on a lighter stain and at the same time, a mass of dense material makes its appearance in the cytoplasm which stains like chromatin (Figs. 40, 41 and 45). Koltzoff ('06) and Binford ('13) regard this material as mitochondria. I think this is nuclear material which has diffused out into the cytoplasm as the chromatin content has become reduced. I found a similar condition of affairs in the spermatids of *Cancer magister* (Fasten, '18).

Soon a vacuole makes its appearance at one end of the cytoplasm, the nucleus wanders towards the opposite pole and the centrosome with the mitochondria-like mass take the middle position between them (Figs. 42, 43, 46 and 47). In the spermatid which contains the chromatoid body, all changes go on as usual, but the chromatoid body wanders to the periphery of the cytoplasm and is ultimately eliminated from the cell (Figs. 39–43), thus playing no further part in the transformations. From now on the spermatids seem to be similar and the same changes take place in them.

The mitochondrial mass soon becomes ring shaped and the centrosome occupies its center. The upper end of the nucleus has penetrated the open inner space of the mitochondrial ring as seen in Figs. 48 and 49. The karyosome-like granule has also wandered upward until it comes to lie directly below the centrosome. The spermatid now appears like Fig. 49.

Subsequent to this stage there is a fusion between the centrosome and the karyosome-like body of the nucleus to form a single structure. This then elongates to look like a short rod (Fig. 50). Going hand in hand with these changes, a bubble makes its appearance at the upper end of the first vacuole (Fig. 50), which gradually increases in size (Figs. 50–55) and becomes the second vacuole. In *Lophopanopeus bellus* a careful study of the smear preparations reveals the fact that this second vacuole was formed through a diffusion of substance from the distal end of the central rod. This is clearly shown in the figures

indicated. The second vacuole increases in size and ultimately surrounds completely the central body so that the spermatid presents the picture illustrated by Fig. 55. In this figure the primary and secondary vacuoles have changed into distinct vesicles and from now on they will be designated as the primary and secondary vesicles.

Soon a thin line grows out from the distal portion of the central body and this penetrates the upper end of the second vesicle to form a small opening (Fig. 56). The central body then begins to hollow out thereby becoming tubular. At the same time the outer opening of the second vesicle becomes collar-like in appearance. These changes are seen in Figs. 57–59. Following this the nucleus and mitochondrial ring fuse and become like one structure drawn up tightly around the vesicles (Fig. 60). From this last named nuclear-mitochondrial portion, the radial arms of the mature spermatozoa are formed (Fig. 61).

The last transformation in the mature spermatozoa occurs in the central body. The proximal end of this structure penetrates the tubular, distal end to form a small spine-like vertical rod (Figs. 62 and 63). This can only be seen in slides of spermatozoa which have been greatly destained. In other slides the spermatozoa all present the appearance of the one shown in Fig. 64. When such a male gamete that has been stained with Heidenhain's hæmatoxylin and acid-fuchsin is examined, the central body is uniformly black except for its distal end where it joins the dark collar-like opening, which is clear; the second vesicle is an amber color; the primary vesicle is colorless, and the nuclear-mitochondrial mass is darkly granular, with a darker ring immediately around the second vesicle. Such mature spermatozoa fill in the lower portion of the testis and the vas deferent ducts.

When these spermatozoa begin to open up one notices that four types of them exist, a three (Fig. 65), four (Fig. 66), five (Fig. 67) and six (Fig. 68) rayed type. The four and five rayed types predominate in largest numbers. The opening up and explosion of these spermatozoa has already been described in a previous contribution to this journal (Fasten, '21). As pointed out in that paper, the explosion is produced by a lowering in the osmotic pressure of the medium which surrounds the gametes.

Summary.

1. During the latter part of June and early portion of July the testis of *Lophopanopeus bellus* (Stimpson) Rathbun is in the best condition for the study of the spermatogenesis process.

2. Primary and secondary spermatogonial divisions can be distinguished. The spermatogonial chromosomes are univalent and probably number around 124.

3. Large nutritive cells are frequently associated with spermatogonial strips in tubules where there are mature spermatozoa. These have irregular nuclei, and are, undoubtedly, produced from primary spermatogonia which have failed to mature.

4. The primary spermatocyte undergoes growth, parasynapsis, tetrad-formation and reduction division. There are 62 bivalent chromosomes seen in polar views of the metaphase stages of this division.

5. During the growth period a chromatoid body appears in the cytoplasm, and when the reduction division takes place this wanders undivided to one of the poles of the cell, resulting in the formation of two kinds of secondary spermatocytes, one which possesses the structure and the other which is devoid of it.

6. There is no rest period between the primary and secondary spermatocytes. The division of the latter is equational and produces two types of spermatids, one having a chromatoid body and the other which is minus such a structure. This last type is about three times as numerous as the former one.

7. At an early stage, the chromatoid body is expelled from the spermatids which contain it, and from then on all the spermatids undergo similar complicated transformations. These changes bring about the formation of the radial spermatozoa which are packed away in single spermatophores within the vas deferent ducts.

8. Four kinds of mature spermatozoa may be distinguished, namely, three-, four-, five- and six-rayed types. The four- and five-rayed spermatozoa are the ones which are encountered most frequently.

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EXPLANATION OF PLATES.

The figures in the accompanying plates were drawn with the aid of the camera-lucida at a magnification of 1,600 times. They are all from smear preparations.

EXPLANATION OF PLATE I.

FIG. 1. Resting primary spermatogonium.

FIG. 2. Fragmentation of chromatin into smaller clumps, primary spermatogonium.

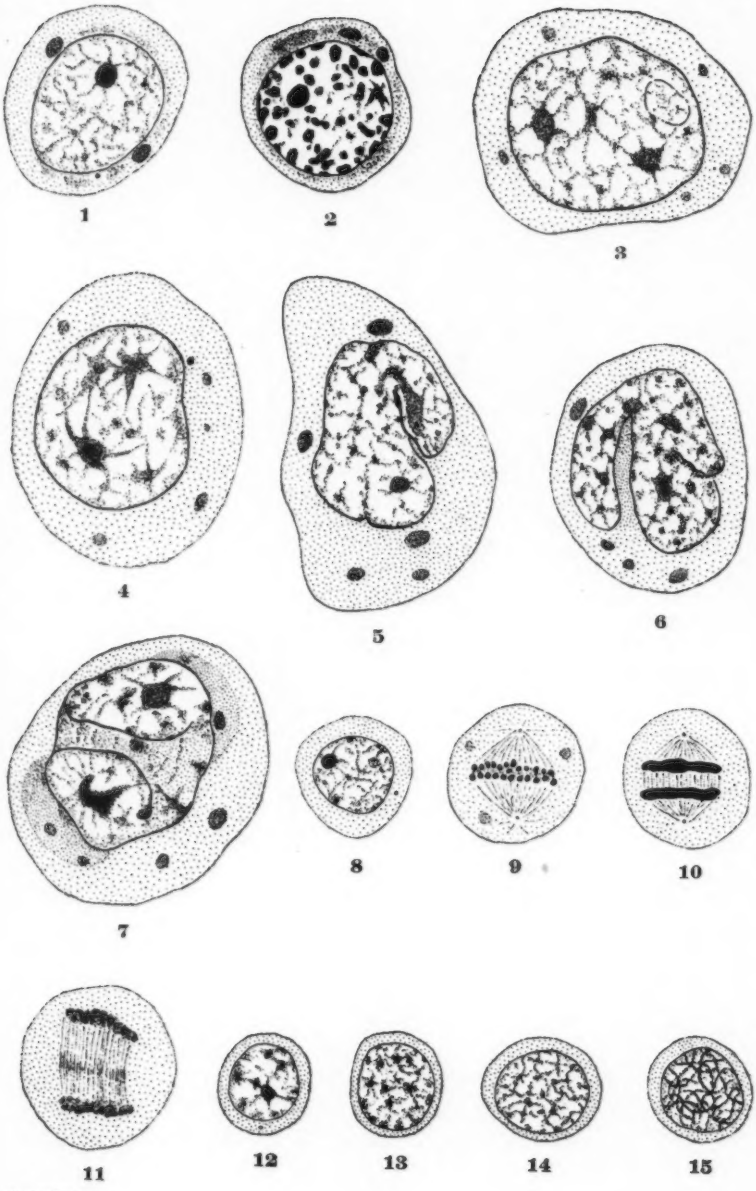
FIGS. 3 TO 7. Nutritive cells.

FIGS. 8 TO 11. Secondary spermatogonia in various stages of mitosis.

FIG. 12. Resting primary spermatocyte.

FIGS. 13 AND 14. Early growth stages, primary spermatocyte.

FIG. 15. Leptotene stage, primary spermatocyte.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Explanation of Plate II.

Fig. 16. Pre-synapsis stage, primary spermatocyte, showing the parallel, side-by-side arrangement of pairs of leptotene threads.

Fig. 17. Pachytene stage, primary spermatocyte.

Figs. 18 and 19. Diplotene and post-diplotene stages, primary spermatocyte.

Figs. 20 and 21. Pre-tetrad, tetrad and dumb-bell formation, primary spermatocyte.

Figs. 22 and 23. Metaphase stages, primary spermatocyte.

Fig. 24. Polar view, metaphase, primary spermatocyte, showing sixty-two bivalent chromosomes.

Figs. 25 to 28. Stages in division of primary spermatocyte.

Figs. 29 to 34. Stages in division of secondary spermatocyte that possesses a chromatoid body.

Figs. 35 to 38. Stages in mitosis of secondary spermatocyte which lacks the chromatoid body.

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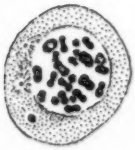

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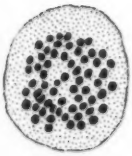

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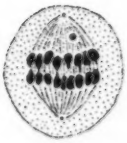

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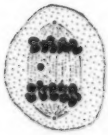

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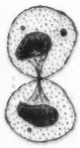

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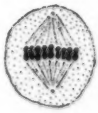

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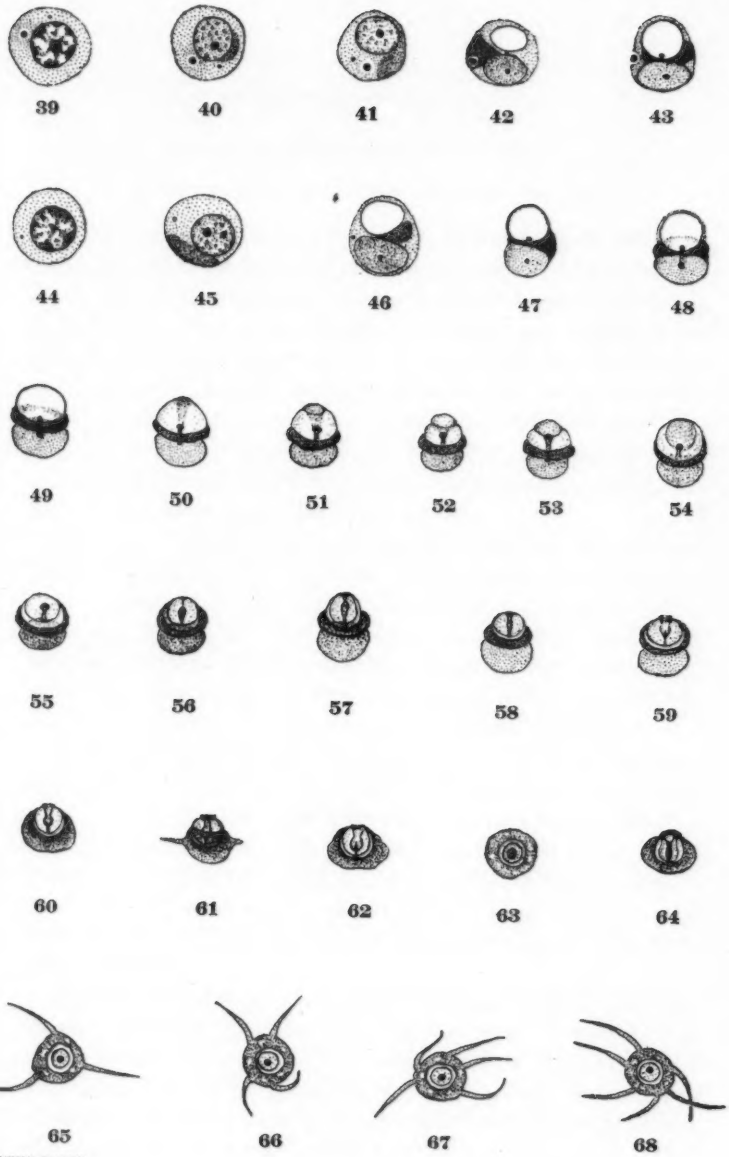
Explanation of Plate III.

Figs. 39 to 43. Stages in transformation of the spermatid that contains a chromatoid body. In Fig. 43 the chromatoid body is expelled.

Figs. 44 to 47. Stages in the transformation of the spermatid that does not possess a chromatoid body.

Figs. 48 to 64. Later stages in transformation of both types of spermatids.

Figs. 65 to 68. The four types of spermatozoa produced.



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SOLE PATTERNS OF TWINS.

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In the study of twins, one frequently desires to know whether or not they are of monozygotic or dizygotic origin. This question can be answered by examination of the placenta and membranes at birth, but when older children and adults are studied it is difficult, and often impossible, to do so. Work has been done on the general physical appearance, mentality, palm patterns, whorls of the hair, etc., but no definite data has been secured. This study was undertaken in the hope that the friction-ridge patterns on the sole of the foot might reveal some clue as to the type of twin from whom they were secured.

Newman [1, 2] thinks that monozygotic twins are apt to have identical patterns, that they are found nearly always in this type of twin, but that unlike patterns do not disprove their monozygotic origin. Wilder [3] also has remarked upon the striking similarity which sometimes occurs. He [4] states that while they are similar grossly, the Galton details are always different. Accurate information on this subject is lacking.

Prints were secured from 87 pairs of twins living in Madison and vicinity; the process perfected by Professor Mathews being used in order that the fine details might be more readily studied. The youngest pair was one week old, and the oldest 85 years. Most of the subjects, however, were school children. Thirty-three pairs were male-male, 24 were female-female, and 30 were male-female. Of the 174 individuals examined, 96 were male and 78 female.

The ratio of monozygotic to dizygotic twins has been variously stated. Newman [1] thinks that the monozygotic type occurs about 25 per cent. of the time, while De Lee [5] quotes Ahlfeld as stating that 15.55 per cent. of all twins are monozygotic. Ahlfeld's figures are, perhaps, more reliable because he examined the placentas and membranes in 1,157 twin births.

The Wilder method is used in classifying the patterns and

a summary of his method is as follows: The pattern bearing area on the ball of the foot is divided into two parts, one proximal to the great toe (hallucal) and the other proximal to the smaller toes (plantar). When three ridges meet to form a "Y" it is called a delta of which there are three in the hallucal area, one distal (just below the great toe), one medial, and one lateral. If all three of them are present the pattern is a whorl (W), if the distal one is absent the resulting pattern is a loop opening toward the great toe and is designated as an "A" pattern, if the medial one is absent and the loop opens medially a "B" pattern is formed, and if the lateral delta is not present a "C" pattern results. When no pattern is present it is called an open field (O). The plantar area contains three places in which a pattern may be found. These lie in the three interdigital spaces proximal to the four smaller toes. Four general types of patterns are found in these areas: the open field or no pattern, designated by an O, a loop opening distally (U), a loop opening proximally (∩), and a whorl (W). To illustrate the application let us turn to the right foot of twin 8 (Fig. 1). The hallucal pattern is a loop opening distally and is described as an 'A' pattern. The first plantar area also contains a loop opening distally but is designated by a 'U,' as is the second plantar area. In the third area there is no pattern (O). Bringing the various symbols together we have AUUO. To simplify matters, Wilder has proposed a table in which each combination of plantar patterns is given a number: O O O = 1, O O U = 2, O O ∩ = 3, etc. The combination here is 21, and the formula is thus abbreviated to A21. Proximal to the plantar areas there are frequently found other deltas which are designated by the symbol 'd,' and as there are two in this print, we arrive at the formula A21dd. To formulate the left foot we proceed in the same manner except that the plantar patterns are still read from left to right, giving the formula A6dd. The formula of the right foot is placed as the numerator of a fraction and the left foot as the denominator: $\frac{\text{A21dd}}{\text{A6dd}}$.

In order that a set might be called identical the following conditions must be met: (1) either all four patterns, or both

right and both left patterns must have the same formula, and (2) there must be no marked differences in the patterns even if they have the same formula. As space does not permit a more detailed description of the Wilder method of classification, the reader is referred to his writings on the subject. Fig. 1 illustrates

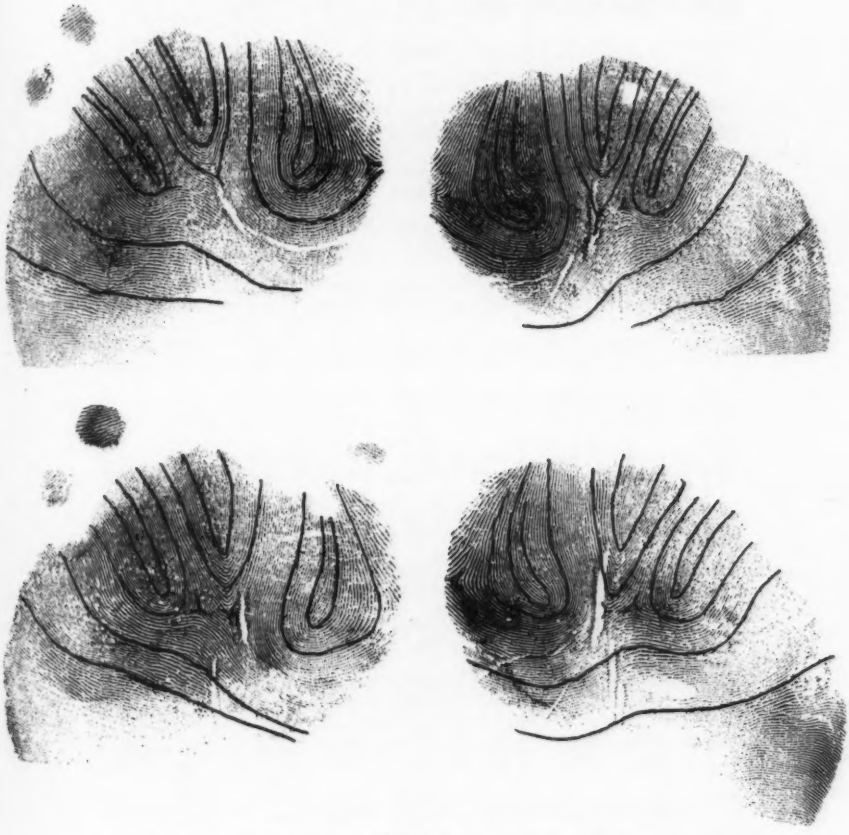

FIG. 1. Twins 7–8. Formula: $\frac{A21dd}{A6dd}$. Some of the lines have been inked in order that the patterns might be more readily seen.

a set of identical patterns. On close examination the similarity of the patterns of each individual is striking. In this series 13 sets (14.94 per cent.) are identical (Table II.), and all except

one occurred in like-sex twins. The striking points of similarity in the different sets are enumerated below. Table I. gives the complete list of patterns. Although twins 25–26 have the same formula, they are not classed as identical because of the very rudimentary character of one of the patterns and so do not meet the conditions stated above.

TABLE I.

COMPLETE LIST OF ALL TWIN PATTERNS.[1]

No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.
1	♀	W38dd	W23dd	59	♀	A5d	A5d	117	♀	W5d	B5d
2	♂	A1	A1	60	♀	A5d	A21dd	118	♀	W7d	W1
3	♂	A37d	A5d	61	♂	A5d	A5d	119	♂	A6dd	A21dd
4	♂	A5d	A1	62	♂	A1	A5d	120	♂	A6dd	A21dd
5	♂	W25d	B5d	63	♀	A17d	A1	121	♀	A1	A1
6	♂	B5d	B5d	64	♂	W13d	W13d	122	♂	A1	W3
7	♀	A21dd	A6dd	65	♂	A5d	A1	123	♂	A6dd	A21dd
8	♀	A21dd	A6dd	66	♂	A5d	A5d	124	♂	A6dd	A5d
9	♀	W14ddd	W13d	67	♂	A5d	A5d	125	♂	A5d	A5d
10	♀	W12	W5d	68	♂	A5d	A1	126	♀	A6dd	A1
11	♂	B5d	A5d	69	♂	A1	A1	127	♀	A5d	A11
12	♀	A1	A1	70	♀	A37d	A7d	128	♀	W5d	A5d
13	♀	A29dd	A6dd	71	♀	A37d	A7d	129	♂	O45d	O15
14	♀	A5d	A5d	72	♂	A57d	A1	130	♀	A13d	W15d
15	♂	W5d	W5d	73	♀	A6dd	W5d	131	♂	O1	A1
16	♂	W5d	W5d	74	♀	W5d	W5d	132	♀	A5d	B1
17	♂	A5d	A5d	75	♀	B1	B1	133	♂	A1 *	A1
18	♂	A37d	A7d	76	♀	B1	A1	134	♀	A1	A1
19	♀	W5d	W5d	77	♀	B45d	W7d	135	♀	W5d	W5d
20	♀	W5d	W5d	78	♀	B45d	W7d	136	♀	A1	A1
21	♀	A6dd	A1	79	♀	W1	W1	137	♀	A1	A1
22	♂	W33	W1	80	♀	A5d	A3	138	♀	B5d	A5d
23	♂	A37d	A5d	81	♀	W6dd	W5d	139	♂	A5d	A7d
24	♂	A5d	A5d	82	♀	A5d	W5d	140	♂	W5d	W5d
25	♂	W5d	W5d	83	♂	W49d	W4d	141	♂	W5d	W5d
26	♀	W5d	W5d	84	♂	W61dd	W12d	142	♀	A2d	A7d
27	♀	A5d	A5d	85	♂	A5d	A5d	143	♀	W1	W3
28	♂	W45d	W15d	86	♂	A5d	A5d	144	♀	A37d	W1
29	♂	A5d	A1	87	♀	A45d	A9	145	♂	B1	B1
30	♂	A5d	A1	88	♂	A45d	W13d	146	♂	A33	A1

TABLE I.—(*Continued.*)

No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.
31	♀	A5d	A5d	89	♀	A5d	A5d	147	♀	A2d	A5d
32	♂	B14dd	B29dd	90	♀	W1	W5d	148	♂	A37d	A5d
33	♂	W5d	W5d	91	♂	A5d	A5d	149	♂	W13d	A43
34	♀	A37d	A3	92	♂	A52dd	A6dd	150	♂	W41d	W15d
35	♂	A1	A1	93	♂	W2d	W21dd	151	♂	A5d	A5d
36	♂	A1	A1	94	♀	B6dd	B21dd	152	♂	A5d	A5d
37	♂	A1	A1	95	♂	A5d	A1	153	♂	A5d	A5d
38	♀	A5d	A1	96	♀	B6dd	B5d	154	♂	A6dd	W5d
39	♀	B38dd	B7d	97	♀	A1	W1	155	♀	O38d	O5
40	♂	W37d	W7d	98	♂	A5d	A5d	156	♀	O46d	A15d
41	♂	A6dd	A21dd	99	♀	A33	W9	157	♀	A17d	B1
42	♂	A5d	A7d	100	♀	W45d	W11	158	♂	W5d	W5d
43	♂	W46dd	W7d	101	♂	A1	W1	159	♀	W1	W1
44	♂	A45d	W15d	102	♂	A5d	W5d	160	♀	A5d	A5d
45	♂	A5d	W1	103	♂	W1	W1	161	♂	A33	A2d
46	♂	A1	A1	104	♂	W1	W1	162	♂	W5d	B5d
47	♂	A2d	A17d	105	♂	W5d	W5d	163	♂	W5d	W5d
48	♂	A5d	A5d	106	♂	B1	B5d	164	♂	B6dd	B23dd
49	♀	W5d	A5d	107	♂	A5d	O5	165	♂	A1	W1
50	♀	A5d	A5d	108	♂	W37d	W7d	166	♂	A5d	A5d
51	♂	A1	A1	109	♀	A5d	A1	167	♀	A38dd	A1
52	♀	A17d	A2d	110	♀	A5d	A1	168	♀	A6dd	A21dd
53	♂	W37d	W47d	111	♀	O15d	O29d	169	♀	A41	A11
54	♀	A5d	A21dd	112	♂	W57d	W3	170	♀	A41	A11
55	♂	A5d	W5d	113	♀	A1	A5d	171	♂	A37d	A3
56	♀	W37d	W7d	114	♀	A1	W1	172	♂	A1	A5d
57	♂	A5d	W7d	115	♂	W13d	W5d	173	♂	A38dd	A63dd
58	♀	W13d	W47d	116	♂	W37d	W7d	174	♀	A5d	A7d

[1] In this table ♂ means male, and ♀ means female. The twins are grouped in pairs.

Twins 7–8.—Note the odd shape of the hallucal pattern "A" in both right feet and the pointed character of the first plantar pattern in all four feet. The plantar patterns of the "U" type are usually rounded. The ridge counts from the cores of the hallucal patterns to the cores of the first plantar patterns are: 7R, 37; 7L, 38; 8R, 38; 8L, 39. From the first to the second plantar patterns the count is: 7R, 28; 7L, 30; 8R, 27; 8L, 29.

TABLE II.

THIS GIVES ONLY THOSE PATTERNS WHICH ARE IDENTICAL.[2]

No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.
7	♀	A21dd	A6dd	103	♂	W1	W1
8	♀	A21dd	A6dd	104	♂	W1	W1
15	♂	W5d	W5d	109	♀	A5d	A1
16	♂	W5d	W5d	110	♀	A5d	A1
19	♀	W5d	W5d	119	♂	A6dd	A21dd
20	♀	W5d	W5d	120	♂	A6dd	A21dd
29	♂	A5d	A1	133	♂	A1	A1
30	♂	A5d	A1	134	♀	A1	A1
35	♂	A1	A1	151	♂	A5d	A5d
36	♂	A1	A1	152	♂	A5d	A5d
77	♀	B45d	W7d	169	♀	A41	A11
78	♀	B45d	W7d	170	♀	A41	A11
85	♂	A5d	A5d				
86	♂	A5d	A5d				

Twins 15–16.—Although this is a common pattern, all hallucal patterns are counter-clockwise, and all except one are of the lateral pocket variety.

Twins 19–20.—Here, too, all hallucal patterns are counter-clockwise. The ridge counts from the hallucal to the second plantar patterns are: 19R, 73; 19L, 72; 20R, 83; 20L, 80.

Twins 29–30.—This is a common pattern. The prints are too blurred to do a ridge count accurately.

Twins 35–36.—All show finer lines in the plantar areas although there are no patterns present.

Twins 77–78.—Both right feet and both left feet have the same pattern. The plantar areas are of a type not usually seen.

Twins 85–86.—There are suggestions of patterns in the third plantar areas on all four feet.

Twins 103–104.—All hallucal patterns are of the seam variety.

Twins 109–110.—Both right feet and both left feet have the same pattern. The left feet (and not the right) have fine lines in the plantar areas although there are no patterns present.

[2] In this table ♂ means male, and ♀ means female. The twins are grouped in pairs.

Twins 119–120.—The general type of patterns found are not a very common variety. The ridge counts from the second to the third plantar areas are: 119R, 39; 119L, 37; 120R, 27; 120L, 25.

Twins 133–134.—In this set the twins are of opposite sex. This type of pattern is of common occurrence.

Twins 151–152.—The plantar patterns on both left feet have a "V" shape.

Twins 169–170.—This type of pattern is uncommon.

An attempt was made to count the ridges from the various landmarks, but they vary so greatly in most instances that it was not thought worth while to report all of the figures. The striking similarities are given above.

The individual patterns occur in almost the same ratio as those found in a previous series.[6]

The fact that twins 133–134 are of opposite sex might lead one to conclude that study of the sole prints is without value in determining the type of twin under consideration, but I feel that such is not the case. They are of the A1 type, one of the simplest and commonest. I have found that A1 patterns occur about 10 per cent. of the time on both feet, and so do not think it unreasonable to assume that their presence in this set of twins is an accident.

In order to determine whether or not patterns of the same formula might be found on all four feet of ordinary (not twin) brothers and sisters, the prints of 38 pairs (the only ones available at this time) were examined. Of these 10 were female-female, 12 male-male, and 16 female-male. One pair of sisters had the same patterns, but they were of a very common type, A5d.

Conclusions.

A study of the above data leads one to the conclusion that the presence of identical patterns on the soles of a pair of twins might point to their monozygotic origin, but as Newman states, their absence does not disprove it. A study such as this is, of course, not conclusive, but it points the way for further investigation. It was undertaken in the hope that sole patterns might reveal some information as to the origin of twins. If the

placentas and patterns of a large series could be examined at birth, the question would be answered.

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SPERM FILTRATES AND DIALYZATES.

Their Action on Ova of the Same Species.[1]

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Introduction.

The idea persists that spermatozoa bear a specific substance which is essential in fertilization. Yet all attempts to extract from spermatozoa a substance which will cause development of ova of the same species have failed or have been open to grave criticism. It is now possible to avoid certain errors which invalidated the results of these investigations. The experiments, to be reported here, were undertaken to determine whether, by processes of filtration and dialysis, solutions can be obtained from living sperm which will effect activation and development of ova of the same species. For interesting me in this problem and for invaluable aid in its solution I am indebted to Dr. Otto C. Glaser.

The experimental work was carried on during the summers of 1919–1922 at the Marine Biological Laboratory at Woods Hole, Massachusetts, and from December until June 1920–1921 at the

[1] Thesis submitted in partial fulfillment of the requirements for the degree of Ph.D. at the University of Michigan. Contributions from the Department of Zoölogy, Smith College, No. *138*.

Hopkins Marine Laboratory at Pacific Grove, California. I wish to express my appreciation of the hospitality extended to me at these laboratories, and my thanks to their Directors, Dr. Frank R. Lillie and Dr. Walter K. Fisher, for their aid and encouragement.

The fact that the spermatozoa, rather than the medium which carries them, are the essential agents in fertilization was established in 1824 by Prevost and Dumas; and it is now conceded that fertilization is monospermic and involves the combination of nuclear material from a single spermatozoön and a single ovum. In many species only a single spermatozoön penetrates the cytoplasm of the egg, as was shown by Hertwig (1876); but even when in normal fertilization more than one enters the egg, as in the pigeon, the pro-nucleus of only one enters into the formation of the fertilization nucleus (Harper, 1904, and Blount, 1909). As stated by Glaser (1915), "a single spermatozoön is sufficient to carry on the biparental effect."

It is well known, however, that in the process of fertilization in any species the spermatozoa far exceed the ova in number. This was first noted in frogs by Spallanzani (1785), and verified by Prevost and Dumas (1824). Under such conditions direct contact of every ovum with a single spermatozoön is possible. But mere contact does not ensure fertilization. Even in physiological solutions spermatozoa rapidly lose their fertilizing power, Vernon (1899); Gemmil (1900); Dungay (1913); Lillie (1915) and Cohn (1918). Two explanations have been offered in recent years to account for the excess of spermatozoa. Lillie (1915) implies that in an excess at least one spermatozoön *possessing sufficient fertilizing power* ("sperm receptors") will reach each ovum. On the other hand Glaser (1915) and Cohn (1918) state that the extra spermatozoa may produce changes in an ovum which facilitate the inclusion of a single spermatozoön.

The idea of a superficial effect of sperm on ova is supported by results obtained with *Nereis limbata*, Lillie (1911) and Goodrich (1920); with *Arbacia punctulata*, Lillie (1921); with *Sabellaria alveolata* and *Halosydna gelatinosa*, Labbe (1921, 1922); and with *Asterias*, Chambers (1923). There are indications that a chemical substance or substances are produced by the sperm:

Godlewski (1911); Herlant (1912); Heilbrun (1915); and Lillie (1911, 1915, 1921).

What real evidence have we to support the suggestion, recurrent in one form or another, that the sperm exude a substance which facilitates fertilization? The early attempts of Spallanzani (*loc. cit.*), with filtrates, and of Prevost and Dumas (*loc. cit.*), with filtrates and extracts of spermatozoa, to demonstrate such a substance gave purely negative results. In later work: Pierri (1899); Winkler (1900); Du Bois (1900); Gies (1901); Pizon (1905); Jacoby (1910); Morse (1912); Robertson (1912); Foa (1918)—positive results, where reported, are actually vitiated by errors of technique or interpretation, Loeb (1913, p. 104, and 1916, p. 203); and Lillie (1919, pp. 133, 134).

II. Methods.

In an investigation of this kind so much depends upon the methods employed that it seems desirable to present these in some detail.

All glassware was cleaned with potassium-bichromate-sulphuric acid mixture, subsequently removed by washing in fresh and in sea-water. All solutions were made with analyzed chemicals (Kahlbaum's, Merck's, and Baker's) with water redistilled from glass. Specific gravity was determined in early experiments with a standard hydrometer and later with a Westphal balance. A special set of indicators and standards provided and tested at intervals by Hynson, Westcott and Dunning was used for the colorimetric determination of the hydrogen-ion concentration, Clowes and Smith (1923 and 1924). Every possible precaution was taken to avoid accidental insemination and sea-water controls accompanied every experiment.

Method of Obtaining Ripe Reproductive Cells of Sea-urchins.—One method is as follows: The instruments and hands of the operator are thoroughly washed in tap water and the animals are washed in tap water followed by sea-water. They are opened by cutting through the oral disc with sterile scissors, the disc and the alimentary canal are removed with sterile forceps and the cœlom is flushed with filtered sea-water to remove the body fluids and any intestinal contents. Males and females are

placed on opposite sides of the operator. Each animal, unless shedding freely, is immediately wiped and placed on its aboral surface in a Syracuse watch glass. In this position the reproductive cells exude through the genital pores. In a second method suggested by Dr. Glaser, the spines are rubbed off before the animals are washed and dried. If the reproductive elements are ripe, shedding begins immediately and it is unnecessary to cut the "test." There is no admixture with body fluids or with sea-water and very clean dry reproductive cells are obtained. Only ripe spermatozoa are shed and these are collected in a beaker. Not all the eggs shed are ripe. For this reason it is necessary to fertilize a sample of eggs from each female. After this the "certified" eggs are placed together in a finger bowl with sufficient sea-water to keep the concentration of egg-water below a point at which it will injure the eggs.

Method of Obtaining Ripe Eggs of Nereis limbata.—The method recommended by Lillie (1911) and Just (1915*a*) was employed. Males and females as they were caught were segregated in finger bowls of sea-water and kept cool until all preparations for an experiment were complete. The most satisfactory results are usually obtained with reproductive cells taken from such animals within two hours after collection; although, if necessary, shedding can be prevented for twelve hours or more if the dishes containing the animals are kept on ice. Since either drying the animals on filter paper or washing them in distilled water leads to shedding, each animal was "sterilized" by first transferring it to a finger bowl containing two hundred and fifty milliliters of sea-water. (In this volume of sea-water any sperm adhering to the body of the female will lose their fertilizing power.) Subsequently each animal was placed in a dry Syracuse watch glass in which any excess of moisture was absorbed with strips of filter paper, and there cut transversely with sterilized scissors. The eggs or sperm were forced out by the spasmodic contractions of the body muscles. To avoid accidental insemination the males were opened with a second set of instruments; and all inseminated controls were kept separate from the experiments in which special solutions were being tested. Eggs and sperm were kept covered and cool until used.

Preparation of Filters.—Berkefeld and Mandler diatomaceous filters were used: in preliminary experiments the coarse and medium grade of Berkefeld filters; and in all other work Mandler filters, tested to six to twelve pounds air pressure in the size two and one half by five eighths inches. The latter are used generally in bacteriological work. These were boiled in five per cent. aqueous solutions of sodium bicarbonate, washed and boiled repeatedly in redistilled water until the wash water was neutral in reaction. Finally they were thoroughly washed in filtered sea-water. After this a stream of sea-water was passed through the filters until samples of such water produced no injurious effect on unfertilized ova and no disturbance of fertilization or of development. Such filters were considered "clean." Every filter was subjected to this treatment each time that it was used. The necessity for these precautions was indicated by a variety of experiences. The first sea-water passed through a boiled and washed filter may be sufficiently hypotonic to cause cytolysis of ova within twenty-four hours. Unless all alkali is removed the filtered sea-water may produce activation of ova. The use of acid in the cleaning of filters is prohibitive because the filtrates would then contain traces of heavy metal dissolved from the filter bands. The impurities in question are often too slight for detection by chemical tests, but are only too clearly revealed by their effects upon unfertilized or fertilized ova.

Preparation of Sperm Filtrates.—Five and ten per cent. suspensions of spermatozoa were prepared by adding to a definite volume of dry sperm a measured quantity of sea-water. These were allowed to stand at room temperature (fifteen degrees centigrade at Pacific Grove and twenty-two degrees centigrade at Woods Hole) for from five minutes to four hours. To ensure the greatest activity of the spermatozoa the carbon dioxide generated by them was prevented from accumulating by a thorough aëration of the suspensions. The latter were then either clarified first by centrifuging or by filtering through filter paper, or were transferred directly to a diatomaceous filter. Contamination of the filtrates by back suction was prevented by collecting the filtrates into Pyrex test tubes set in the suction flasks.

During the passage of the filtrate it is necessary to prevent or at least to minimize the destruction of spermatozoa by dehydration or compression. This was attempted by not allowing the surface of the mantle to become exposed to air and by frequent and cautious stirring of the suspension. Some of the spermatozoa are not seriously injured in the process of filtration as indicated by the isoagglutinable and fertilizing power of the sperm remaining on the surface of the mantle at the end of the process.

The filtrates, which are automatically freed from any excess of carbon dioxide in passing through the filter mantle, are then transferred to flasks which are kept tightly plugged and placed in a refrigerator. These solutions remain free from bacteria and retain their peculiar physiological properties for at least a month.

Preparation of Sperm Dialyzates.—There is one objection to the use of sperm filtrates. Some spermatozoa may undergo destruction on the surface of the mantle. To meet this objection I prepared dialyzates of sperm in order to compare their action on ova with that of filtrates.

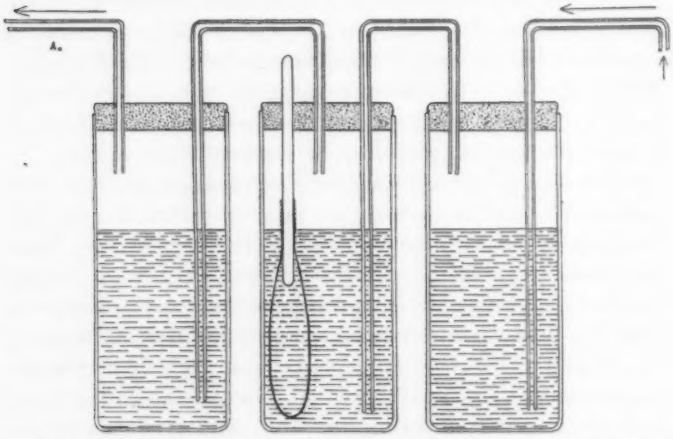


FIG. 1. Dialyzing apparatus. Air is drawn through the sea-water in the three bottles by suction exerted at *A*. The collodion sac is suspended in the sea-water in the central bottle and this sea-water dialyzate used in experiments later. The outer two bottles serve as safety flasks.

Collodion sacs used as dialyzers were made in fifty milliliter centrifuge tubes from a twelve per cent. collodion prepared

according to the method of Gates (1921). The sacs were washed in redistilled water and in sea-water. Samples of sea-water, allowed to stand in the washed sacs for at least twelve hours, were tested in the same manner as that passed through the diatomaceous filters. Tests for leakage were made and any imperfect sacs were discarded. Ten milliliters of twenty-five or of fifty per cent. suspensions of sperm were placed in a tested collodion sac and dialyzed against forty milliliters of sea-water. The resultant dialyzates may be considered comparable to filtrates prepared from five and ten per cent. suspensions of sperm. The sea-water dialyzates must be aërated constantly to provide the most favorable conditions for the sperm during the process. This involves two dangers: evaporation and contamination of the dialyzates. In order to eliminate these sources of error the method illustrated in Fig. 1 was employed.

In these experiments dialysis was continued for five to twenty-four hours. With one exception the sperm in the collodion sacs retained their fertilizing power and their capacity for iso-agglutination at the end of the process.

III. Properties of Preparations.

a. Physical and Chemical Properties.—The osmotic pressure of filtrates and dialyzates, as indicated by the specific gravity tests, is the same as that of sea-water. There is a slight variation in some preparations and in sea-water but it is not sufficient in itself, as determined by experimentation, to produce activation. The hydrogen-ion concentration of filtrates and dialyzates was also equal to that of sea-water (p_H 7.9–8.1 at Woods Hole; 7.6–7.8 at Pacific Grove), indicating that the carbon dioxide formed by active sperm was completely removed by aëration and that the sperm added no other free hydrogen-ions. There is a possibility that small amounts of acid may have combined with buffers in sea-water. The question now arises whether these preparations contain any active physiological principle. No living spermatozoa or fragments were ever observed, nor was there ever a case of normal fertilization in any of the preparations. It is also certain that they contain no chemical substance whose concentration falls within the range of sensitivity of the usual chemical methods.

Experiments in which blood plasma was passed through a Berkefeld filter, Cramer and Pringle (1913), and Goddard (1914), demonstrated that the filter held back for a time, not only suspended elements, but the various proteins in colloidal solution, but the third portion passed through the filter contained some fibrinogen. Mudd (1922) suggested that in alkaline solution protein may be carried through in small amounts. The filter itself is negatively charged and because of the amphoteric properties of protein the latter dissociates as an anion in alkaline solutions and so would tend to be carried through the filter.

It is important therefore to determine if possible whether nitrogen compounds are present since Loeb (1914) states that protamine will induce the first cell divisions in eggs of *Arbacia;* and Labbe (1923) using a one to forty thousand solution of sodium nucleinate in sea-water on unfertilized eggs of *Arbacia* obtained some swimming larvæ. Because of buffer action these solutions had the same hydrogen-ion concentration as sea-water, and Labbe concluded that the sodium nucleinate exerted a specific action not due to its hydrogen-ion concentration.

All the usual tests for protein or protein split products such as guanine, protamine, and nucleic acid were negative. A micro-kjehldahl test, performed for me by Dr. W. Dennis, was also negative, indicating that nitrogen is not present in sufficient amounts to be detected by this method. Incineration tests gave evidence of the presence of carbon in larger amounts in both filtrates and dialyzates than in sea-water.

b. Physiological Properties.—From the presence of carbon in these preparations it is certain that they contain something of organic origin. It seemed possible that they might produce changes in the ova of foreign species, since sperm extracts obtained by other investigators have produced marked effects on ova of foreign species even when they had no influence on ova of the same species, Loeb (1916, p. 102). Consequently tests were made to determine whether *Arbacia* sperm filtrates would affect the ova of *Nereis limbata*, a form in which maturation follows insemination. Seventeen experiments were performed in which ten preparations were used on *Nereis* eggs. The results obtained resemble in many respects those produced by in-

semination with *Nereis* sperm. The foreign filtrate causes production of jelly, the formation of a "fertilization membrane," complete maturation, and segmentation leading to the development of larvæ.[2] The latter however are abnormal in shape and in the distribution of cilia. In no case did these changes occur in sea-water controls. A protocol of one experiment is given below.

TABLE I.

CHANGES IN THE OVA OF *Nereis* EXPOSED FOR TWENTY MINUTES TO A FILTRATE PREPARED FROM A TWO PER CENT. SPERM SUSPENSION OF *Arbacia*.

Date.	Exper.	Per Cent. of Eggs Forming Jelly and Membranes.	Per Cent. of Dividing Eggs.	Per Cent. Swimming.
8/ 4/20	31	100	10	0.5
8/ 5/20	33	99	90	0
8/ 7/20	35	81	75	5
8/ 8/20	37	85	85	9
8/ 8/20	38	100	14.5	2
8/13/20	40	100	1	0
8/14/20	43	100	5	2
8/14/20	44	100	13.5	1
8/14/20	45	100	12	0.5
7/27/21	225	33	31.5	1
7/27/21	225	30	28.5	1

In experiments 31, 40, and 43 the ova were stale. In all cases membrane formation and maturation occurred as rapidly in ova in sperm filtrates as in inseminated ova. Subsequent development in ova treated with filtrate was delayed.

The *Arbacia* sperm filtrate acted like a parthenogenetic agent, yielding results similar to those obtained in *Nereis* with other methods (Lillie, 1911, and Just, 1915).[2] I obtained similar results with filtered egg-water of *Arbacia*. The latter observations, quoted by Dr. Alvalyn Woodward (1921), were verified frequently in subsequent experiments. Further experimentation demonstrated that the effect produced by sperm filtrates on *Nereis* eggs bears a definite relation to the strength of the preparations and to the duration of exposure of ova to them. The main question however is what effect have such filtrates and dialyzates on ova of the same species.

[2] The developing ova divided into two, four and eight cells, and subsequently some of them developed into abnormal ciliated trochophores. The rest cytolyzed.

IV. The Potency of Sperm Filtrates and Dialyzates on Ova of the Same Species.

a. Arbacia punctulata.—The procedure employed to determine the effect of these preparations on ova of the same species and the results obtained are described first for *Arbacia*. The ova were exposed to the action of sperm filtrates and dialyzates in the proportion of two milliliters of fresh washed eggs to twenty-five milliliters of the test solution. Sea-water controls accompanied every experiment and samples of eggs from test solution and from sea-water were examined at intervals.

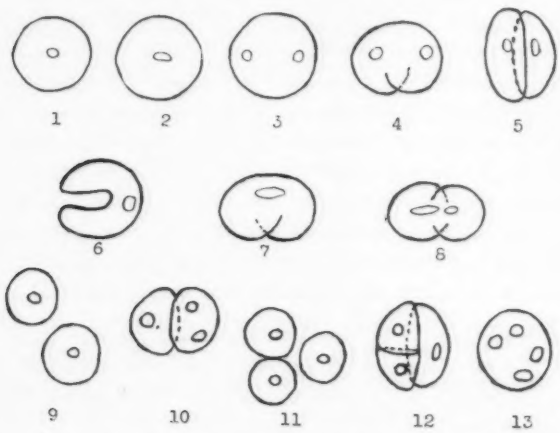
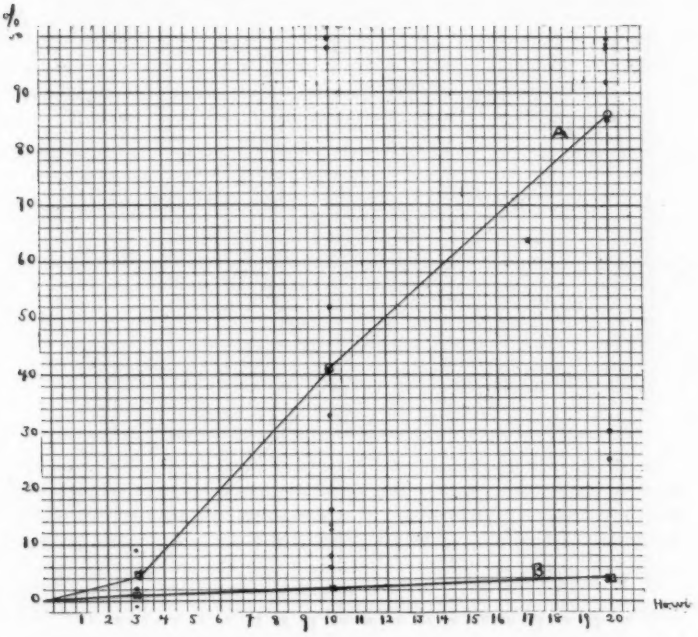


Fig. 2. Changes in shape and nuclear changes in ova exposed to sperm filtrates. 1–5 = changes in a single ovum within forty-five minutes; 6–13 = ova representing various changes produced by filtrates. Magnification × 350.

The first evidence of the influence of the filtrate or of the dialyzate on the ova is a change in the density of the cytoplasm at or near the center of the ovum, similar to that which precedes nuclear division in inseminated eggs. Indeed nuclear changes and nuclear division follow. Simultaneously a distortion of the egg occurs followed by its partial or complete cleavage into two or more parts. The cleavages are at times perfectly regular, at times unequal. Even the smallest cells are usually nucleated. Segmentation sometimes proceeds to the eight-celled stage, but in any case is followed, if the eggs remain in the filtrate, by a

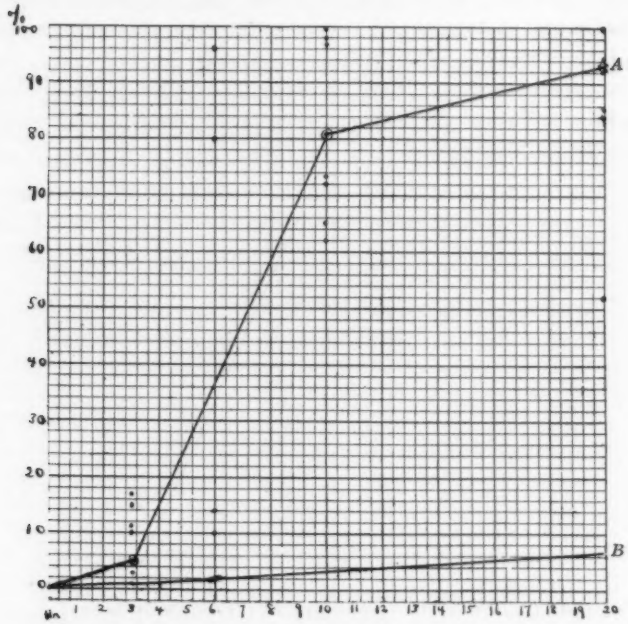
separation of the parts and their ultimate cytolysis. In a very few cases a slightly elevated membrane is visible. These results were reproduced with different lots of eggs and with different filtrates and dialyzates. In no case did these changes occur in sea-water controls or in sea-water filtered or dialyzed. The latter results indicate that no substance is given off by the filters or collodion sacs that can account for the effects of sperm filtrates and dialyzates.



GRAPH I. The percentage of ova divided and cytolyzed by sperm filtrates of the same species—*Arbacia punctulata*. *A* = the average of ten experiments with filtrates made from 2 per cent. suspensions. *B* = the average of ten sea-water controls. ● = percentages in individual experiments with sperm filtrates.

Confirmation of these results was obtained in a series of forty-two experiments with filtrates and twenty-four experiments with dialyzates. Within twenty-four hours the majority of ova in the test solutions had undergone decided changes, while those in

controls remained normal in appearance. This is well illustrated in the percentages indicated in graphs I., II., and III.[3]



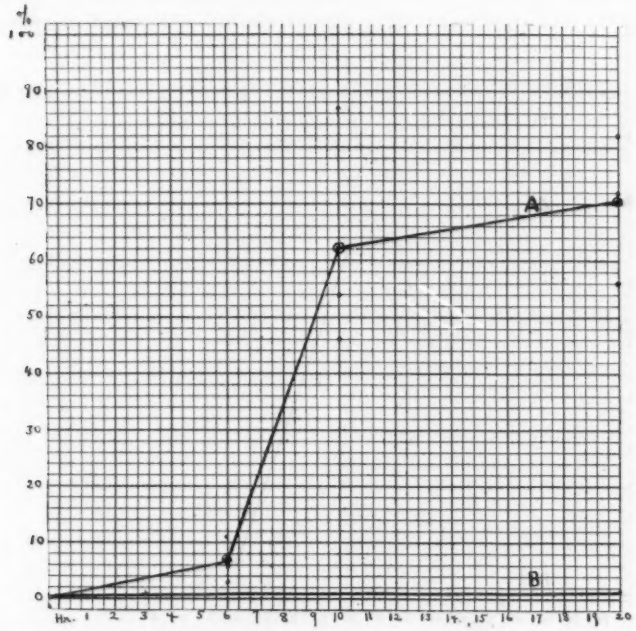
GRAPH II. The percentage of ova divided and cytolyzed by sperm filtrates of the same species—*Arbacia*. *A* = the average of ten experiments with filtrates made from 5 per cent. suspensions. *B* = the average of ten sea-water controls. ● = percentages in filtrates in individual experiments.

It is evident that some ova, probably because of a slight difference in physiological condition, are more rapidly affected by the filtrates and dialyzates than others, thus indicating a varying degree of susceptibility of the eggs to the action of the preparations.

In view of the failure of other investigators of this problem it is important to emphasize the fact that I obtained consistent results. At the same season of the year the eggs of different

[3] The graphs indicate the number divided and cytolyzed rather than simply the ones in a state of division. Since the ova do not divide simultaneously, some have divided and cytolyzed at a time when others are undergoing nuclear changes or are dividing.

females are about equally susceptible, and filtrates or dialyzates prepared in the same manner are equally effective on ova of the same individual, as indicated in graphs IV. and V.



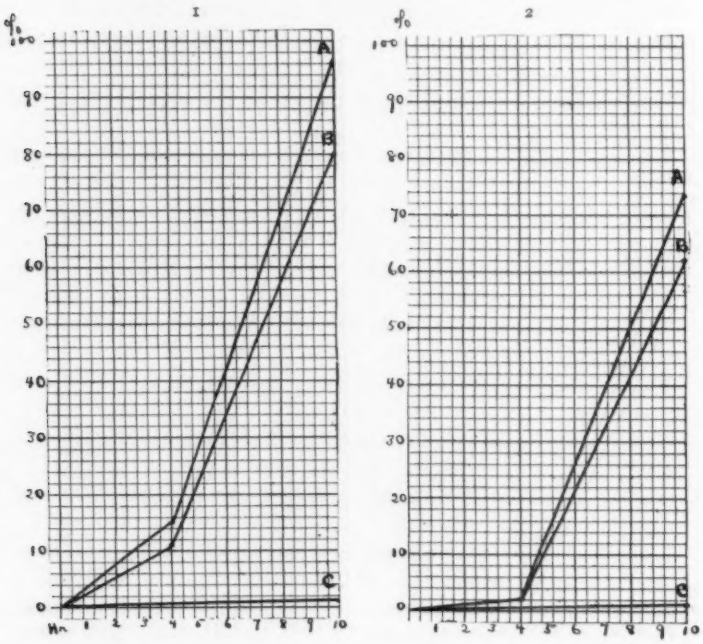
GRAPH III. The percentage of ova divided and cytolyzed in sperm dialyzates of the same species—*Arbacia punctulata*. *A* = the average of three experiments with dialyzates from 50 per cent. suspensions. Duration of dialysis = ten hours. *B* = the average of three experiments with sea-water dialyzed for ten hours against sea-water. ● = percentage in dialyzates in individual experiments.

It is apparent that, if the eggs are in the same physiological condition and are exposed at the same temperature to filtrates of equal strength, one may expect equal amounts of segmentation and cytolysis in approximately equal lengths of time.

As might be expected a definite correlation exists between the strength of the test solutions and the percentage of eggs affected in a given time. This is well illustrated in graphs VI., VII., VIII., and IX.

The definite correlation which exists between the duration of

exposure and the percentage of eggs affected suggested experiments in which the eggs are removed from the filtrate before the latter have had opportunity to produce any visible changes in the eggs. If now there are invisible effects and if these are orderly and significant, they should become noticeable after the

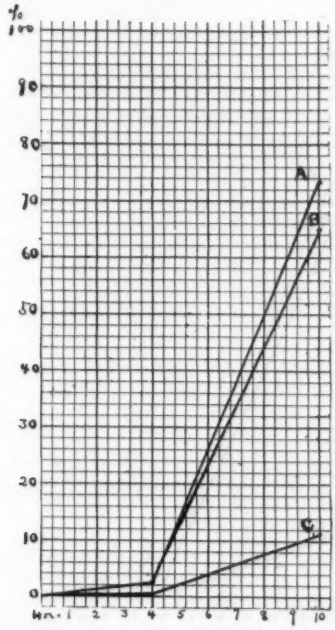


GRAPH IV. Percentage of division and cytolysis of ova produced by sperm filtrates on two sets of eggs—*Arbacia punctulata*. 1 = Filtrate 6/30/21 from a 5 per cent. suspension. 2 = Filtrate 7/2/21 from a 5 per cent. suspension. *A* = one set of eggs. *B* = second set of eggs. *C* = control eggs in S.W.

eggs are transferred to sea-water. Cases in point are illustrated in graphs X., XI., and XII., and indicate clearly that the gross visible effects, distinct only after several hours, are preceded by important changes which may develop in some of the ova within a very few minutes. These changes may, after transfer of the ova to sea-water, lead to progressive changes in the ova.

The Effect of Filtrates Prepared from Heated Sperm.—The statement was made by Winkler (1900) and by Morse (1912)

that when sperm suspensions were heated to 50°–60° centigrade the solutions failed to affect eggs. I therefore made filtrates from suspensions of *Arbacia* sperm heated to 42°–50° centigrade to compare their action with those of filtrates from unheated



GRAPH V. Percentage of ova divided and cytolyzed by two similar filtrates of sperm of the same species—*Arbacia*. *A* = Sperm filtrate 6/30/21 from a 5 per cent. suspension. *B* = Sperm filtrate 7/2/21 from a 5 per cent. suspension. *C* = Sea-water control.

sperm suspensions. The spermatozoa lose their iso-agglutinable and their fertilizing properties at a temperature of 38°–40° centigrade and tend to adhere to one another. One heated suspension was first passed through filter paper and then through a Mandler filter. As a control a part of the same suspension, unheated, was similarly treated. A comparison of the effect of the two filtrates is given in the following table.

TABLE II.

A COMPARISON OF THE ACTION OF FILTRATES PREPARED FROM HEATED AND FROM UNHEATED SPERM SUSPENSIONS OF *Arbacia* FIRST PASSED THROUGH WHATMAN FILTER PAPER.

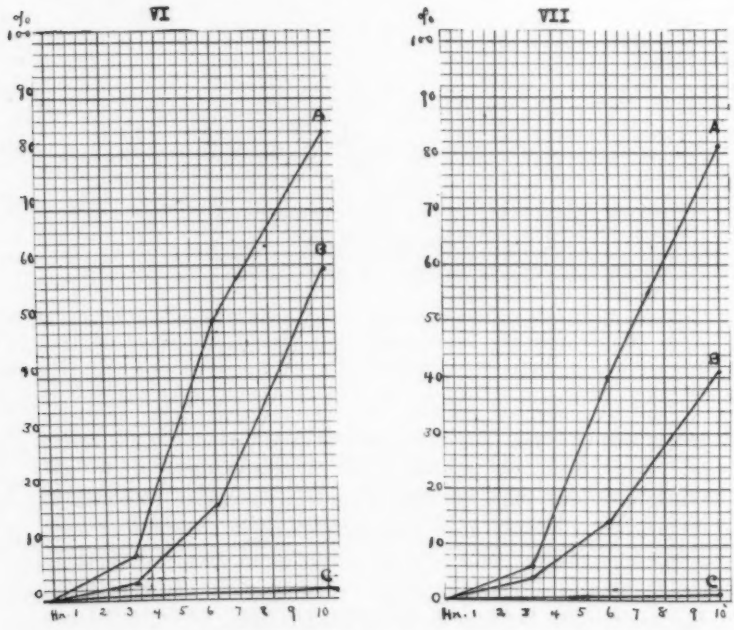
Filtrate from	Per Cent. of Ova Affected. 4.5 Hrs.				Per Cent. of Ova Affected. 7.5 Hrs.			
	Nor.	Abn.	Div.	Cyt.	Nor.	Abn.	Div.	Cyt.
I. Fresh Sperm.....	74	24.5	0.5	1	1	0	0	99
II. Heated Sperm.....	99	0	0	1	99	0	0	1

Nor.—normal undivided ova.

Abn.—undivided ova, abnormal in shape and in the appearance of the cytoplasm and nucleus.

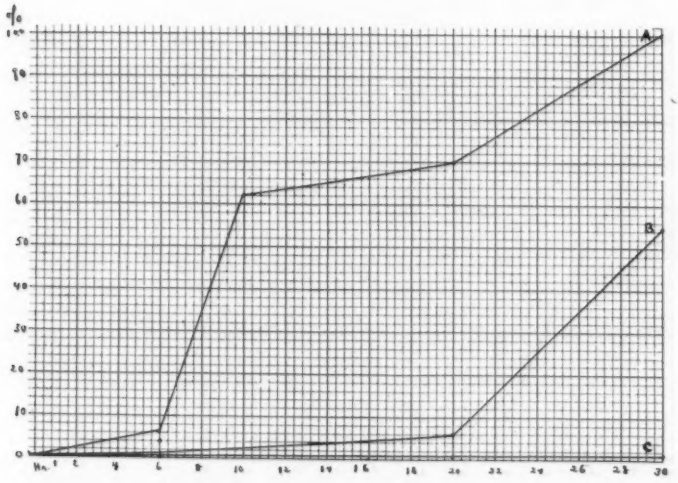
Div.—divided.

Cyt.—cytolyzed.



GRAPHS VI. AND VII. The relation of the strength of the filtrate to the percentage of ova divided and cytolyzed—*Arbacia*. VI. Average of four experiments. VII. Average of twelve experiments. *A* = sperm filtrate from a 5 per cent. suspension. *B* = sperm filtrate from a 2 per cent. suspension. *C* = sea-water control.

Practically no heated sperm reach the surface of the filter mantle as they do not pass through the Whatman filter paper. This might appear to indicate that any active substance is adherent to the heated coagulated sperm and not readily given off into sea-water.



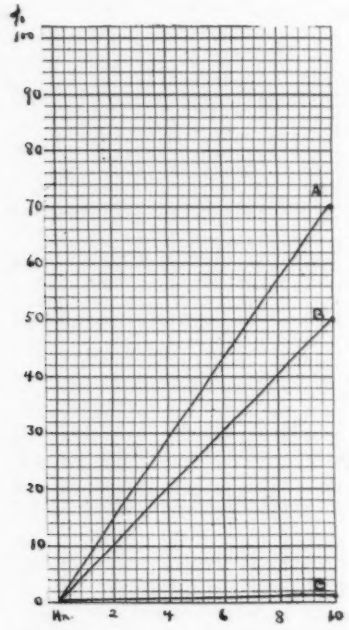
GRAPH VIII. Relation of the strength of the dialyzate to the percentage of ova divided and cytolyzed—*Arbacia punctulata*. *A* = average of three experiments with dialyzates from 50 per cent. sperm suspensions. *B* = average of three experiments with dialyzates from 25 per cent. sperm suspensions. *C* = average of six experiments with sea-water controls. Duration of dialysis = ten hours.

Another filtrate was made by filtering a heated sperm suspension directly through a Mandler filter. As a control part of the same suspension, unheated, was passed through a second filter. The effects of these two preparations are compared in Table III.

The filtrates of the heated sperm suspension have as great an effect on unfertilized ova of the same species as filtrates of fresh suspensions if the suspensions are filtered directly.

The Effect of Boiled Filtrates of Arbacia Sperm.—If the substance which so affects the eggs is an enzyme, heated filtrates may be ineffective. A filtrate prepared from a five per cent. sperm suspension was heated to boiling and then cooled rapidly.

An equal volume of sea-water was treated in the same manner and used as a control. Unheated filtrate and unheated sea-water served as additional controls. The results are given in Table IV.



GRAPH IX. Correlation between the duration of dialysis and the percentage of ova divided and cytolyzed—*Arbacia*. *A* = dialyzate from a 50 per cent. sperm suspension. Duration of dialysis = twenty hours. *B* = dialyzate from a 50 per cent. sperm suspension. Duration of dialysis = ten hours. *C* = sea-water dialyzed for twenty hours against sea-water.

Heating the filtrate as above indicated does not greatly affect its power to produce segmentation and cytolysis of ova of the same species. It should be emphasized that the hydrogen-ion concentration and the osmotic pressure of these solutions are like those of the sea-water controls.

It is thus evident that filtrates and dialyzates of spermatozoa of *Arbacia* contain some substance, not destroyed by heat, which produces profound changes in ova of the same species.

TABLE III.

A COMPARISON OF THE ACTION OF FILTRATES PREPARED FROM HEATED AND FROM UNHEATED SPERM SUSPENSIONS OF *Arbacia* FILTERED DIRECTLY THROUGH MANDLER FILTERS.

Exp.	Filtrate from	Per Cent. of Ova Affected. 2 Hrs.			Per Cent. of Ova Affected. 20 Hrs.		
		Nor.	Abn.	Cyt.	Nor.	Abn.	Cyt.
167	I. Fresh Sperm	98	0	2	0	0	100
	II. Heated Sperm	100	0	0	0	0	100
170	I. Fresh Sperm	62	0	38	0	0	100
	II. Heated Sperm	53	0	47	0	0	100

Nor.—normal undivided ova.
Abn.—undivided ova, abnormal in shape and in the appearance of cytoplasm and nucleus.
Cyt.—cytolyzed. (Partial or complete division precedes cytolysis.)

TABLE IV.

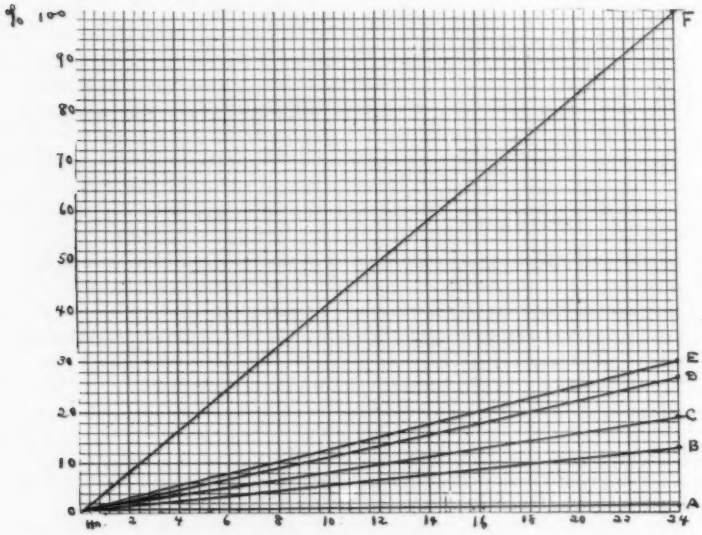
A COMPARISON OF THE ACTION OF HEATED AND UNHEATED SPERM FILTRATES ON OVA OF THE SAME SPECIES—*Arbacia punctulata*.

Time = 14 Hrs.	Per Cent. of Ova Affected. Exp. *A*—III.			Per Cent. of Ova Affected. Exp. *A*—IV.		
Solution.	Nor.	Div.	Cyt.	Nor.	Div.	Cyt.
Sea-water	98	0	2	99	0	1
Heated Sea-water	98	0	2	99	0	1
Sperm Filtrate	62	3	35	74	3	23
Heated Filtrate	84	0	16	81	2	17

Nor.—undivided and normal.
Div.—divided.
Cyt.—cytolyzed.

b. Strongylocentrotus purpuratus and Strongylocentrotus franciscanus.—The same methods employed with two other species of sea-urchins—*Strongylocentrotus purpuratus* and *Strongylocentrotus*

franciscanus—at Pacific Grove, California, from January to June 1921, yielded similar results. Longer exposures were required than with ova of *Arbacia*. The ova of *S. franciscanus* were the more susceptible to the action of the sperm filtrates, but unfortunately ripe ova of this species were rare during these months



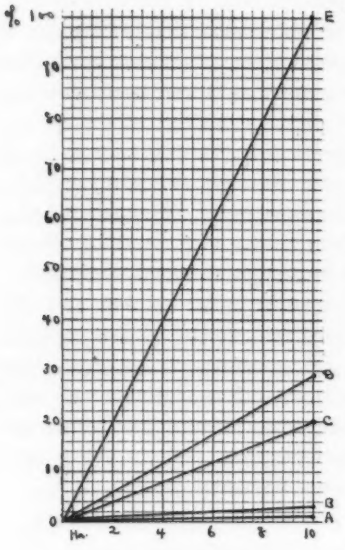
GRAPH X. The effect of limited exposure of ova to sperm filtrates—*Arbacia*. Experiment 156—Observation at the end of twenty-four hours. The percentage of ova divided and cytolyzed is indicated. *A* = sea-water. *B* = exposure to 5 per cent.* filtrate for five minutes. *C* = exposure to 5 per cent. filtrate for ten minutes. *D* = exposure to 5 per cent. filtrate for thirty minutes. *E* = exposure to 5 per cent. filtrate for sixty minutes. *F* = exposure to 5 per cent. filtrate for twenty-four hours.

of 1921 because of an extremely cold season. It is well known that the ova of *S. purpuratus* are resistant to parthenogenetic agents, Loeb (1916, pp. 99–103). The hope that the conditions of low temperature (12°–15° C.) and high hydrogen-ion concentration (p_H 7.6–7.8) prevailing at Pacific Grove might favor normal segmentation and normal development of ova treated with sperm filtrates was not realized. Yet, as will be demonstrated in later experiments, a brief exposure to such filtrates

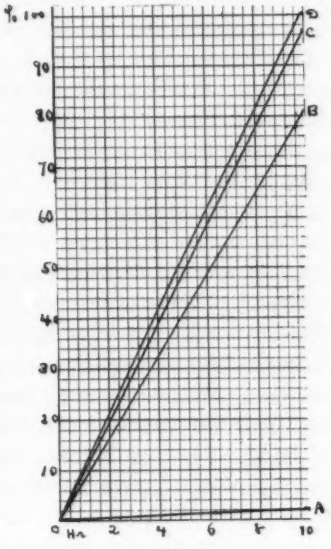
* 5 per cent. filtrate = a filtrate from a 5 per cent. suspension.

produced decided changes in ova of both species of *Strongylocentrotus*.

c. Nereis limbata.—Sperm filtrates of *Nereis limbata* produce effects on ova of this species comparable to those produced by foreign sperm filtrates. An exposure of *Nereis* eggs to filtrates



GRAPH XI.



GRAPH XII.

The effect on ova of *Arbacia* of limited exposure to filtrates.

Filtrate of a 2 Per Cent. Suspension.	Filtrate of a 2 Per Cent. Suspension.
A = sea-water 10 hrs.	*A* = sea-water 10 hrs.
= filtrate 15″–45″	= filtrate 15″–45″
B = " 75″	*B* = " 210″
C = " 135″	*C* = " 4.5 hrs.
D = " 195″	*D* = " 9 hrs.
E = " 9 hrs.	

The percentages of ova divided and cytolyzed are indicated.

made from one, two or ten per cent. *Nereis* sperm suspensions results in the formation of jelly and fertilization membranes and the complete maturation of a certain percentage of eggs. The most satisfactory results were obtained with eggs transferred directly to a two per cent. filtrate, and within two hours after

the *Nereis* were collected. In this experiment the majority of the eggs matured. The maturation was almost as rapid as in fertilized eggs but the subsequent segmentation and development was slow and abnormal. Segmentation into two, four, and eight cells was observed and verified for me by Dr. Alvalyn Woodward. Free swimming trochophores developed which were abnormal in shape, internal structure, and in the distribution of cilia. In no case did such changes occur in the sea-water controls. Similar results were obtained with four sperm filtrates of *Nereis limbata*.

Summary of the Effects of Sperm Filtrates and Dialyzates on Ova of the Same Species.—Sperm filtrates and dialyzates of *Arbacia punctulata*, and sperm filtrates of *Strongylocentrotus purpuratus*, of *Strongylocentrotus franciscanus*, and of *Nereis limbata* produce marked changes in ova of the same species. These are characterized by changes in form; nuclear and cell division; and, with the exception of *Nereis limbata*, by subsequent fragmentation and cytolysis. "Fragmentation" is a separation of the segments due possibly to a lack of a fertilization membrane. In *Nereis limbata* the sperm filtrates cause maturation; formation of fertilization membranes; segmentation; and, in a low percentage of cases, the development of abnormal larvæ.

V. Filtrates as Factors in Parthenogenesis.

It seemed possible in view of the results obtained by Loeb (1905) and others that the ova of sea-urchins, treated with filtrates of sperm, might develop perfectly if they were subsequently exposed for a brief period to Loeb's "hypertonic sea-water". In Loeb's experiments, exposure of unfertilized ova of sea-urchins to hypertonic sea-water, at the temperature prevailing during the breeding season, produced development in a very small percentage of the ova. Fertilization membranes did not form usually and the developing plutei did not swim at the surface of the water as did normal plutei. A brief preliminary treatment of the ova with a very dilute solution of butyric acid increased the percentage of ova which developed, and the latter were normal in macroscopic structure and in their reactions. Recently Just (1922), employing a greater concentration than

that used by Loeb, has succeeded in producing healthy viable plutei of *Arbacia punctulata* by exposing ova of this species to hypertonic sea-water alone. In view of these results it may be that the butyric acid sensitizes the eggs to the action of the hypertonic sea-water. Experiments were undertaken to determine whether or not sperm filtrates may also act as sensitizers.

The procedure employed in this phase of the investigation was as follows: unfertilized eggs of *Arbacia* were exposed for periods of one minute to two hours to a sperm filtrate and subsequently for twenty to thirty-five minutes to Loeb's hypertonic sea-water. A control series consisted of ova exposed first to sea-water and then to Loeb's hypertonic sea-water. One set of unfertilized and one set of fertilized eggs served as controls for each experiment.

In the majority of experiments no development took place in the ova exposed to hypertonic sea-water only. When such an exception occurred, the percentage of ova which developed was small and the plutei did not swim at the surface of the water. In every experiment many of the ova subjected to the double treatment, *i.e.*, to the filtrate followed by the hypertonic sea-water, developed as far as the blastula and gastrula stages. A small percentage developed into perfect plutei which swam at the surface. The majority cytolyzed. Because of the high percentage which cytolyze at the time when the blastulæ and gastrulæ form, it is impossible to determine the exact percentage which develop to these stages. The sea-water containing them was centrifuged and the residue examined. An effort was made to ensure the transfer of equal quantities of eggs and of sea-water in each experiment, and equal periods of centrifuging were employed to ensure equal possibilities of precipitation of ova or of developing individuals. The results of these experiments are given in Table V.

As already indicated the sperm filtrates rarely cause membrane formation. In the few instances where these form, they are thin and but slightly elevated. In one filtrate such membranes developed on ova after an exposure to the filtrate for thirty to thirty-five minutes. Of the ova remaining in the filtrate for one to fifteen minutes and subsequently transferred to hypertonic

sea-water for twenty to thirty-five minutes, a larger percentage developed than in other experiments. In this series no ova developed after exposure to hypertonic sea-water alone. In Table VI. the results of these experiments are summarized. The figures indicate approximate percentages.

TABLE V.

A COMPARISON OF THE NUMBER OF OVA OF *Arbacia* WHICH DEVELOP AFTER TREATMENT WITH FILTRATE FOLLOWED BY EXPOSURE TO HYPERTONIC SEA-WATER.

Time of observation and total count = 70 hrs.

Transfer from	Sea-water.			Hypertonic S.W. 30″			Hypertonic S.W. 35″		
	B.	G.	P.	B.	G.	P.	B.	G.	P.
Sea-water 15″	0	0	0	1	0	1	2	0	0
Filtrate									
2″	0	0	0	13	0	5	56	0	28
10″	0	0	0	0	0	2	41	0	14
15″	0	0	0	74	0	0	36	0	3

The letters B, G, and P indicate blastulæ, gastrulæ, and plutei respectively. The figures given here indicate the number, not the percentage, of developing ova.

A comparison of the effects of the preliminary treatment with sea-water and with sperm filtrates reveals, in the latter experiments, a slight increase in the percentage of ova which develop into plutei perfect in macroscopic appearance and in reactions; and a decided increase in the percentage which cytolyze after exposure to hypertonic sea-water. The preliminary treatment with the sperm filtrate apparently sensitizes the ova to the action of the hypertonic sea-water.

Tests were also made to determine whether the sperm filtrates increase the susceptibility of the ova of *Strongylocentrotus purpuratus* to the action of hypertonic sea-water. Certain preliminary experiments were performed to familiarize the writer with the effect of hypertonic sea-water alone on the ova of this species. Ova were exposed to it for periods of thirty minutes to four hours. The effect was tested by transferring them to sea-

TABLE VI.

THE EFFECT PRODUCED ON OVA OF *Arbacia punctulata* BY SPERM FILTRATES OF THE SAME SPECIES FOLLOWED BY HYPERTONIC SEA-WATER.

Transfer from	To Hypertonic S.W. 20″.				Hypertonic S.W. 25″.				Hypertonic S.W. 30″.				Hypertonic S.W. 35″.			
	Nor.	Div.	Dev.	Cyt.	Nor.	Div.	Dev.	Cyt.	Nor.	Div.	Dev.	Cyt.	Nor.	Div.	Dev.	Cyt.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Sea-water 15″	95.8	0	0	4.2	91	1	0	8	90	0	0	10	90	0	0.5	9.5
Filtrate 1″	75.5	0	0	24.5	53.6	0	5.7	41.7	75	0	4.5	20.5	27.5	0	1.5	71
5″	0	0	0	100	75	0	3.8	21.2	62	0	3	35	28.3	0	2	69.7
10″	66	0	3.4	30.6	8.8	0	0	91.2	21.8	0	6.1	72.1	23.6	0	0.4	76
15″	48	0	2.7	49.3	14	0	1	85.0	3	0	0	97	1	0	0	99

water and to inseminated sea-water. Ova treated for thirty minutes to two hours were uninjured. Longer exposures caused cytolysis of ova transferred to sea-water and prevented normal fertilization and development of ova subsequently inseminated in sea-water. The percentage of the ova affected bore a direct relation to the duration of the exposure to the hypertonic sea-water.

A repetition of Loeb's method of producing parthenogenesis in this species demonstrated that treatment with 1.5 per cent. butyric acid for one minute followed by hypertonic sea-water for one hour led to development of the majority of the ova. Other experiments indicated that a slight decrease in hydrogen-ion concentration (*e.g.*, p_H 8.3–8.7) facilitated the process. In place of the butyric acid ova were given a preliminary treatment with sperm filtrates in the hope of obtaining development, or of intensifying the action of the hypertonic sea-water. The results at the end of eighteen hours are indicated in Table VII.

Table VII.

Effect of Exposure to Filtrate Followed by Hypertonic Sea-water on Ova of *Strongylocentrotus purpuratus*.

Exp.	Prep.	% Filt.	Duration of Exposure. To		Effects on the Ova. Percentage.			
			Filtrate	Hypertonic S.W.	Nor.	Abn.	Div.	Cyt.
83	1/26	10	0″	70″	99	0	0	1
			30″	70″	98	0	0	2
			60″	70″	98	0	0	2
			120″	70″	66	0	1	33
			30″	120″	99	0	0	1
			60″	120″	0	97.5	0	2.5
			120″	120″	0	0	0	100

It has already been mentioned that ova of *Strongylocentrotus purpuratus* are more resistant to parthenogenetic agents than are those of *Arbacia punctulata*. In these experiments ova of the former species, exposed either to hypertonic sea-water alone

or to filtrate and hypertonic sea-water, failed to develop. Occasionally atypical membranes formed after the double treatment and irregular division ensued. It is of interest that ova which had been exposed to the filtrate for two hours did not shrink when transferred to hypertonic sea-water, as did those transferred directly from sea-water to the hypertonic sea-water. This would seem to indicate that the permeability of the ova exposed to the filtrate had increased. Another indication of the effect of the filtrate in these experiments follows: Ova given the double treatment of filtrate followed by hypertonic sea-water were less capable of normal fertilization and development than those exposed to hypertonic sea-water alone.

Summary of the Function of Filtrates as Factors in Parthenogenesis.—Exposure of ova of *Arbacia punctulata* or of *Strongylocentrotus purpuratus* to sperm filtrates of the same species produce changes which render them more susceptible to the action of hypertonic sea-water. This is indicated in *Arbacia* by a larger percentage of development and of cytolysis; and in *S. purpuratus* by a greater tendency of ova to cytolyze or to lose their power of normal fertilization and development if exposed to both filtrate and hypertonic sea-water.

VI. Filtrates as Factors in Fertilization.

The increase produced by sperm filtrates in the susceptibility of *Arbacia* ova to parthenogenetic agents, and the apparent increase in permeability of ova of *S. purpuratus* suggest that such preparations may also facilitate fertilization.

An attempt was made to obtain "resistant" eggs early in the breeding season in order to try the effect of fertilizing such ova in sperm filtrates. During two seasons such "resistant" eggs were not found. The ova obtained were either immature, as indicated by their failure to develop if fertilized with a small or large amount of sperm suspension, or else ripe. In the latter case no increase over the usual percentage of development could be expected.

Conditions leading to increase in permeability of mature ova tend to allow the entrance of more than a single spermatozoön when such eggs are inseminated. This polyspermy usually

results in abnormal development. It may occur if mature ova are inseminated in sperm filtrates. To determine this, two milliliters of washed *Arbacia* eggs were placed in twenty-five milliliters of sperm filtrate and, as a control, a similar quantity in twenty-five milliliters of sea-water. These were inseminated immediately, or after exposure to the filtrate or sea-water for from one minute to four hours. Frequent observations were made to determine whether the filtrates affect either fertilization or development. The effects observed are as follows in the ova fertilized in the filtrate and allowed to develop in it: lack of a normal fertilization membrane; abnormal changes in shape; a low percentage of dividing ova; abnormal division; delayed and abnormal development; and cytolysis. In the controls normal fertilization and development occurred. Similar results were obtained in thirteen series of experiments in which several filtrates were used. Gemmil (1900) describes similar conditions resulting from heavy insemination and finds them accompanying polyspermy as demonstrated by histological examination.

The abnormal development which occurs when ova are inseminated in the sperm filtrates may be a result of an abnormal fertilization reaction. If this is due to changes in the ova in the filtrate, it may be that such changes are reversible if the exposure to the filtrate is of short duration. Ova of *Arbacia punctulata* were exposed to filtrates made from two per cent. suspensions for from one minute to two and a half hours, and to filtrates from five per cent. suspensions for one to thirty minutes. Some were inseminated in the filtrates and others after transfer to sea-water. Few of those inseminated in the filtrate developed normally. The majority of those inseminated after transfer to sea-water formed fertilization membranes and developed normally. The changes produced in the ova by the filtrates, which tend to prevent normal reactions between ova and sperm, are reversible if the period of exposure is brief. Prolonged exposure to filtrates, however, wrought such changes in the majority of ova that they lost their capacity for fertilization and development completely.

Ova of *Strongylocentrotus purpuratus* exposed for short periods to sperm filtrates may not exhibit any change in optical appear-

ance or in shape yet may be so influenced that after transfer to sea-water they fail to develop normally if inseminated with fresh sperm. Such was found to be the case in eighteen experiments in which ova were exposed to the action of a number of filtrates and subsequently inseminated in sea-water. Although the sperm were active in the filtrates, few membranes formed and these were abnormal in that they were irregular and but slightly raised from the surface of the egg. Subsequent divisions were irregular; development was slow and abnormal; and the majority of ova cytolyzed later. Gastrulæ formed in a few, but these lacked an enteron, and plutei were irregular in shape with thickened areas not normally present. These resembled plutei obtained when ova are inseminated with a large excess of sperm when polyspermy is known to occur.

A correlation between the reversibility of changes in ova produced by abnormal constituents in sea-water and the duration of their exposure to these substances has been recorded by Loeb (1915) for butyric acid and hypertonic sea-water; by Lillie (1921) for copper; and by Clowes and Smith (1923 and 1924) for hydrogen ions. In some instances the change is of such a nature that it acts as a block to the entrance of sperm; in others it permits polyspermy. Just (1923) found that eggs of *Echinarachnius* fertilized in blood, though they fail to develop, nevertheless take in sperm.

As stated by Clowes (1924) "it is difficult to distinguish polyspermic from abnormally dividing eggs without cytological examination." Such a study of ova of *Arbacia* inseminated in the sperm filtrates reveals that polyspermy occurred in many of the ova.

The interference with the fertilization process may be due in part to injury to the sperm caused by the sperm filtrate. However, examinations of suspensions of sperm in filtrates reveal that the sperm remain active in such suspensions for hours. Furthermore if such sperm are used for insemination of ova in sea-water, they will effect normal fertilization and development. The results tabulated below indicate that the sperm are not injured by an exposure of one hour either to sea-water or to a sperm filtrate in the concentrations employed; but that ova

were affected by a similar duration of exposure to some of the same filtrate.

TABLE VIII.

A COMPARISON OF THE ACTION OF FILTRATES ON THE FERTILIZING CAPACITY OF SPERM AND OF OVA OF *Strongylocentrotus purpuratus*.

Exp.	Prep.	Filt.	Expos.	Ova from Sea-water.				Ova from Sperm Filtrate.			
73	12/28/1	10%		% Nor.	% Div.	% Abn. Div.	% Cyt.	% Nor.	% Div.	% Abn. Div.	% Cyt.
Sperm exposed to sperm filtrate..			0″	0	90	10	0	0	90	10	0
			1″	0	94	3	3	0	59	35	6
			5″	0	95	5	0	0	85	10	5
			10″	0	91	5	4	0	68	29	3
			20″	0	95	3	2	0	56	43	1
			30″	0	96	3	1	3	23	18	56
			60″	0	98	2	0	0	0	30	70
Sperm suspension in sea-water....			30″	0	98	1	1	0	0	33.4	66.6
			60″	0	96	0	4	0	0	12	88

It is also possible that filtrates may interfere with the development of fertilized ova. This proved to be true. The ova of *Arbacia punctulata* and of *Strongylocentrotus purpuratus* transferred ten minutes after insemination in sea-water to sperm filtrates failed to develop normally, and within eighteen hours the majority had cytolyzed.

Summary of the Action of Filtrates as Factors in Fertilization.—Sperm filtrates produce changes in the eggs of the same species which interfere with a normal fertilization reaction if the eggs are inseminated in the filtrate. The changes produced by a brief exposure are reversible; by a longer exposure irreversible. Such filtrates prevent normal development of eggs previously inseminated in sea-water. The changes are of such a nature that the entrance of sperm is facilitated and polyspermy results.

VII. TEST OF FILTRATES FOR A "SPERM-FERTILIZING AND AGGLUTINABLE SUBSTANCE."

Lillie (1919) has suggested that the spermatozoa bear a fertilizing substance identical with the agglutinable substance

which is apparently lost by spermatozoa in staling in sea-water. If this is present in sea-water filtrates and dialyzates, it should combine with the "agglutinin" of "egg-water." This was tested as follows. The agglutinating unit strength of *Arbacia* egg-water was first determined, Lillie (1914). Dilutions of such egg-water with sperm filtrates and with sea-water were compared as to their power to agglutinate fresh sperm suspensions and no difference could be detected in the capacity of the two sets of dilutions to agglutinate sperm. Either an insufficient amount of the combining substance is present in the sperm filtrates or the substance or substances present do not have the power to combine with the agglutinin of the egg-water.[4]

The agglutinin does not pass through collodion sacs. If the substance in sperm dialyzates is the substance postulated by Lillie, egg-water dialyzed against a sperm suspension should lose its agglutinating power more rapidly than a similar egg-water dialyzed against sea-water. In experiments devised to test this theory no difference in the rate of loss of agglutinin could be detected.

The "fertilizin" of Lillie in *Arbacia* egg-water may, according to Woodward (1918), consist of two parts: an agglutinating and an activating substance. The latter only passes through a Mandler filter. This activates sperm of the same species, and has the power of causing parthenogenetic development of ova of *Nereis limbata*, Sampson (unpublished, quoted by Woodward) and Woodward (1921). If its action is intensified by a fertilizing substance given off by sperm, a combination of filtrates of sperm and of egg-water should be more effective than either alone. No such intensifying effect could be demonstrated in any of the experiments devised to test this possibility.

VIII. Discussion.

In *Nereis limbata* and in sea-urchins the fertilizable period of the gametes is short. Causes for the brevity of this period have been discovered for ova but not for spermatozoa, as indicated by Lillie and Just (1924) in their recent survey of the subject.

[4] It should be recalled that some of the sperm remaining on the surface of the filter mantles and in the dialyzing sacs are agglutinable and retain their fertilizing power at the end of the periods of filtration and of dialysis.

The fact that loss of fertilizing power of the sperm occurs rapidly and precedes loss of motility has suggested the idea that the spermatozoön carries a fertilizing substance which may be lost in sea-water; and this has led to various attempts, of which this is the most recent, to isolate such a substance and to produce development of ova of the same species with it. In this investigation filtrates and dialyzates of sperm suspensions in sea-water have been obtained which initiate development of specific ova, although the development is incomplete.

It is not surprising to find that concentrated sperm suspensions exposed to egg-secretions of a foreign species, that extracts of cells, egg-secretions, and blood cause cytolysis of alien ova, "since it is recognized that something present in mammalian blood serum cytolyzes cells of unrelated animals," Loeb (1913). Sperm killed by heat, extracts of cells, and blood serum have no activating effect on ova of the same species. Specific egg-secretion ("egg-water") has no effect on ova of *Nereis*, but according to Glaser (1915) and Woodward (1918) does produce incomplete activation of ova of *Asterias* and *Arbacia*. Prolonged exposure of ova is necessary, and Lillie and Just (1924) have suggested that there are extraneous parthenogenetic factors present in the egg-water employed.

Careful tests of all the sperm filtrates and dialyzates used in this investigation indicate that the preparations do not contain living sperm or fragments of them. The ova employed are not normally parthenogenetic. Controls give evidence that no substance derived from filters or dialyzers is accountable for the results obtained; and factors which might produce parthenogenesis: abnormal specific gravity, abnormal hydrogen ion concentration, and excess of carbon dioxide, are lacking. The preparations contain carbon but insufficient nitrogen to be detected even by microchemical methods. No lipolytic enzyme could be detected. The preparations are not colloidal; and all attempts to obtain precipitates from them by means of alcohol or the reagents used by Robertson (*loc. cit.*) and Woodward (*loc. cit.*) failed. Sperm filtrates and dialyzates activate ova rapidly and this property is not destroyed by boiling. The effect produced is evidently due to a special physiological activator derived from sperm of the same species.

Experiments to determine whether the substance or substances present in filtrates and dialyzates are "tissue specific," acting only on ova, have not been undertaken. It is true that they do not cytolyze species sperm, as the latter retain their fertilizing power after prolonged exposure to such preparations. The latter are not "species specific" since they readily activate ova of unrelated species. Tests have not been performed to determine whether the effect on species and on foreign ova is due to the same constituent of the preparations.

If the substance acts as a superficial cytolytic agent as suggested by Loeb (1916), it is to be expected that membrane elevation or swelling will occur in ova of the same species exposed to the sperm filtrates and dialyzates. Such occurred in *Nereis* only. However, such preparations cause partial activation and changes in the protoplasm of sea-urchin ova; and there is evidence that the properties of the egg surface are affected. Thus, after exposure to sperm filtrates, unfertilized ova of sea-urchins are more susceptible to the action of hypertonic sea-water; are in a condition which facilitates polyspermy; and their permeability is increased. Fertilized eggs, transferred to such preparations within ten minutes after insemination in sea-water, fail to develop normally. This may be due in part at least to an increase in the permeability of the egg surface. The sperm are uninjured by long exposure and the changes in the ova are reversible if the duration of exposure to such preparations is brief.

According to the "fertilizin" theory of Lillie (1914), substances ("receptors") given off by sperm activate "fertilizin" an essential constituent of the cortex of mature eggs. This in turn initiates the development of the egg. If "receptors" exist in active form and in sufficient quantity in these sperm filtrates and dialyzates, the latter should produce the following effects: initiation of development of mature ova of the same species; such activation of "fertilizin" in egg-water as to make the latter an efficient parthenogenetic agent; such combination with an agglutinating substance in egg-water as to destroy the power of the latter to agglutinate fresh sperm suspensions. The first of these results only has been obtained in this investigation. If

sperm receptors are released more readily in specific egg-water, filtrates of sperm suspensions in egg-water may evince greater activating power than a combination consisting of a sperm filtrate and filtered egg-water. Tests gave no indication of any difference between them. It is also possible, as suggested by Lillie (1915), that other substances are extracted from sperm which may tend to neutralize the activating substances released by them.

There is abundant evidence that the sperm in contact with mature ova, or secretions of ova, of the same species undergo changes which are essential for fertilization. Their metabolism is increased as indicated by their increased motility; their chemical composition is changed as indicated by decrease in refringibility, by swelling of the sperm head, by changes in viscosity, and by surface changes which permit agglutination to occur. Spermatozoa may enter unripe eggs which lack "fertilizin" or mature eggs from which it has been removed experimentally but the sperm are not changed and they do not activate the eggs.

It is significant that in order for fertilization to occur *these essential changes in the spermatozoön must be produced when the latter is in close proximity to an ovum.* Sperm which have received a long exposure to sea-water or a brief exposure to egg-water are active and may surround or even enter ova of the same species, yet fail to fertilize them. This loss of fertilizing capacity, the transitory nature of agglutination, and the inability to obtain a second agglutination reaction with sperm are indications of a loss of substances essential in fertilization. To effect perfect development *such substances must be concentrated at the surface of the sperm head at the instant when the latter comes into contact with an ovum.* Under such conditions they may initiate a chain of chemical reactions, starting in the cortex of the ovum and eventually involving all parts of the protoplasm of the egg, *i.e.*, they may activate "fertilizin." If released into sea-water or egg-water normally, or under the experimental conditions of filtration and dialysis, these substances may be unable to produce complete activation of ova of the same species, because of dilution, instability, or neutralization by other substances elimi-

nated or extracted from sperm. They may, however, produce changes in the surface of the ovum which facilitate its reaction with a spermatozoön. These changes may account, in part, for polyspermy when ova are inseminated with an excess of fresh sperm and for the effectiveness of stale sperm if concentrated suspensions are used. Such substances may account for the antagonistic effect produced by sperm of one species on those of another; and for the neutralizing effect of concentrated sperm suspensions on the action of blood serum.

IX. Summary.

1. Solutions obtained by filtration and dialysis of suspensions of living sperm in sea-water activate ova of the same species.

2. Tests indicate that the effect is produced by some substance derived from the sperm, and not by some extraneous parthenogenetic factor.

3. Ova of *Nereis* exposed to specific sperm filtrates form fertilization membranes, complete their maturation and some develop into abnormal trochophores. Ova of sea-urchins fail to form membranes but do undergo nuclear and cell division.

4. Ova exposed to filtrates and dialyzates are rendered more susceptible to the action of hypertonic sea-water, and to the entrance of sperm.

5. In normal fertilization sperm exposed to "fertilizin" undergo profound modification in chemical structure and organization; and unless such modification occurs the sperm fail to fertilize the egg, even though they may enter it. It is possible that substances localized in the surface of the sperm head activate the ovum. Such localization is transitory.

6. Such substances, when given off by sperm into sea-water or egg-water, either because of dilution, decomposition, or admixture with waste products given off by sperm, produce definite changes in such ova, but are unable to effect complete activation of ova of the same species.

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IS THE INSECT METAMORPHOSIS INFLUENCED BY THYROID FEEDING?

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(With 5 Tables.)

The investigations on the influence of the thyroid gland on the rate of metamorphosis in insects have not as yet yielded any decisive results. Northrop ('17) fed larvæ of *Drosophila* on thyroid and ascertained that this gland is not adequate as food for the examined animals. Kunkel ('18) who studied the influence of the thyroid on the development of the fly *Lucilia*, noticed an abbreviation of the pupal stage and a retardation of growth. Abderhalden ('19) irrigated *Euphorbia* plants with 1 per cent. thyroid extract and observed that the caterpillars of *Deilephila euphorbiæ* fed on these plants were "zum Teil auffallend klein." In the researches of Romeis and v. Dobkiewicz ('20) the larvæ of the flies *Calliphora vomitoria* fed on thyroid gland underwent pupation later than the control specimens, their weight, however, being not smaller. From his experiments Kahn ('21) draws the conclusion that in *Corethra plumicornis*, *Ecdyurus forcipula* and *Tenebrio molitor* neither the human thyroid nor the preparation "Jodalbacid" have any influence on the metamorphosis of their larvæ.

The discrepancy of the above results may be explained not only by the heterogeneity of the material of animals and of the substances used, but, at least to the same degree, by the probably too scanty material in separate experiments as well as by the insufficient method of the elaboration of the data recorded. *E.g.*, Abderhalden's opinion mentioned above is not supported by any numerical data and can consequently not be considered as decisive from the biometrical standpoint, all the more as we do not know the pedigrees of the animals under examination.

[1] Paper from the Laboratory of Experimental Morphology., cf. *Mém. de l'Institut National Polonais d'Économie Rurale à Pulawy*, Vol. 5, 1924, presented at print September 22, 1924.

Similarly, we cannot, according to my view, consider as decisive the results obtained by Kahn who relies on 16 *Tenebrio* larvæ of which 6 were kept without any food, and every two specimens from the remaining 10 were fed differently (fresh meat, fresh thyroid, dried thyroid, meal and "Jodalbacid"). The experiments of the same author on *Corethra* and *Ecdyurus* were made on specimens collected in natural waters and therefore on genetically totally unknown materials. Similar relations are often met with also in other problems referring to the glands of internal secretion. In these cases genetically homogeneous animals used for the experiments and statistical elaboration of adequate data, would, according to my opinion, contribute to set aside numerous discrepancies.

My experiments have been made on caterpillars from 4 lots of eggs of *Lymantria dispar* L. deriving from 4 brother-sister matings of moths belonging to the same pedigree and reared by the author for several years. In order to deal with materials homogeneous also in respect to age, I selected exclusively caterpillars which hatched during the night between April 27th and 28th. On June 5th the thoroughly mixed caterpillars of each lot were divided at random in two parts, one of which served as control, the other being fed on leaves with thyroid. Fresh willow twigs were accurately sprinkled with water solution of the "Tablettæ thyreoideæ" from the factory of "Gedeon Richter" in Budapest. Ca. 450 experimental caterpillars received daily: in the beginning 2, from June 12th 3, from June 18th 4 tabloids, equal to 1.0, 1.5 and 2.0 grams respectively of fresh gland. (The efficacy of the preparation had been previously tested on tadpoles of *Rana temporaria*.) The control animals were fed on willow twigs of the same species, sprinkled with pure water. In both cases the leaves were administered daily (after the superfluous water had been dried) in slight excess of requirements, so that the whole food was almost totally consumed by the next day. The thyroid-fed caterpillars took food as readily as those of the control.

All animals were bred in pasteboard boxes of identical size at a temperature of from 14° to 19° C. Each chrysalid being kept separately, the duration of the pupal period could be established

for each specimen. Pupation as well as emergence of moths was checked every day between 6 and 6:15 o'clock in the evening. The chrysalids were weighed immediately afterwards, so that the age of the weighed pupæ ranged from 0 to 24 hours. The data obtained were biometrically studied according to the commonly used formulæ. On account of the remarkable sexual dimorphism the data were collected for either sex separately. The total number of pupæ was 818, of which 234 male and 188 female were thyroid fed specimens and 171 male and 225 female control animals. Emergence occurred from 782 chrysalids, viz. 218, 182, 161 and 221 specimens respectively (cf. the number of animals in separate experiments in Tables I. and II).

The first chrysalis of the whole material appeared on June 24th. The average terms of pupation of the thyroid-fed and of the control caterpillars calculated in relation to the above term of the "first chrysalis" are recorded in Table I. We notice that the observable differences of the terms of control and experimental material were positive only in 3 cases, in the remaining 5 being on the contrary negative. The difference was here biometrically significant exclusively in the males of the second experiment, the ratio of the difference to its probable error in this case only approximating the required number 4. Moreover, on surveying the data referring to the duration of the pupal period (Table II.) we observe that here too the difference between the control and the thyroid-fed animals is not always positive, the ratio of the difference to their probable errors oscillating only between 0.05 and 2.5 and therefore being in no case significant. This indicates that neither the rate of the hystolytical processes in the caterpillars nor the rate of development in the chrysalids were in our experiments influenced by the thyroid added to the food. In other words, this substance did not elicit any changes in the course of metamorphosis of the examined insects.

From the theoretical point of view this result requires however the following remarks. It has often been emphasized that the influence of glands of internal secretion may be not only of various intensity, but even essentially different in relation to the developmental or growth stage of the animals experimented

TABLE I.

DATA CONCERNING THE TERM OF PUPATION OF THE CATERPILLARS OF *Lymantria dispar* L., IN DAYS.

The term of pupation calculated from the day of the appearance of the first pupa in the whole material, *i.e.* from June 24.

n, number of specimens; σ, standard deviation; $A \pm E_A$, average term of pupation with its probable error; $v \pm E_v$, coëfficient of variability of this term together with its probable error; Diff. $\pm E_{Diff.}$, difference together with its probable error; Diff./$E_{Diff.}$, ratio of the difference to its probable error.

Sex.	Number Corresponding to Each Experiment.	Material of Caterpillars.	*n*.	$\pm\sigma$.	$A \pm E_A$.	Difference between *b* and *a* in Averages.		$v \pm E_v$.	Difference between *b* and *a* in Coëfficients of Variability.	
						Diff. $\pm E_{Diff.}$	Diff./$E_{Diff.}$		Diff. $\pm E_{Diff.}$	Diff./$E_{Diff.}$
Males	1	(*a*) "Thyroid"	59	3.17	8.98 ±0.28	−0.36 ±0.36	1.0	35.3 ±2.45	−9.9 ±3.11	3.2
		(*b*) Control.....	45	2.19	8.62 ±0.22			25.4 ±1.92		
	2	(*a*) "Thyroid"	68	2.13	6.21 ±0.17	+0.86 ±0.23	3.7	34.3 ±2.21	−9.3 ±2.76	3.4
		(*b*) Control.....	58	1.77	7.07 ±0.16			25.0 ±1.66		
	3	(*a*) "Thyroid"	50	2.57	10.32 ±0.25	+0.24 ±0.37	0.6	24.9 ±1.78	−0.9 ±2.63	0.3
		(*b*) Control.....	39	2.53	10.56 ±0.27			24.0 ±1.94		
	4	(*a*) "Thyroid"	57	2.61	6.81 ±0.23	−1.09 ±0.37	2.9	38.3 ±2.75	+2.3 ±4.98	0.5
		(*b*) Control.....	29	2.32	5.72 ±0.29			40.6 ±4.15		
Females	1	(*a*) "Thyroid"	55	3.58	13.20 ±0.33	+0.37 ±0.47	0.8	27.1 ±1.87	+2.0 ±2.65	0.8
		(*b*) Control.....	64	3.95	13.57 ±0.33			29.1 ±1.88		
	2	(*a*) "Thyroid"	48	2.29	11.96 ±0.22	−0.81 ±0.32	2.5	19.1 ±1.36	+4.7 ±2.07	2.3
		(*b*) Control.....	59	2.65	11.15 ±0.23			23.8 ±1.56		
	3	(*a*) "Thyroid"	37	3.46	14.92 ±0.38	−0.73 ±0.49	1.5	23.2 ±1.91	+0.4 ±2.52	0.2
		(*b*) Control.....	52	3.35	14.19 ±0.31			23.6 ±1.65		
	4	(*a*) "Thyroid"	48	4.41	11.96 ±0.43	−1.72 ±0.57	3.0	36.9 ±2.87	+2.1 ±4.15	0.5
		(*b*) Control.....	50	3.99	10.24 ±0.38			39.0 ±3.00		

TABLE II.

DATA CONCERNING THE DURATION OF PUPAL PERIOD OF *Lymantria dispar* L., IN DAYS.

n, number of specimens; σ, standard deviation; $A \pm E_A$, average duration together with its probable error; $v \pm E_v$, coëfficient of variability of the duration together with its probable error; Diff. $\pm E_{Diff.}$, difference together with its probable error; Diff./$E_{Diff.}$, ratio of the difference to its probable error.

Sex.	Number Corresponding to Each Experiment.	Material of Chrysalids.	n.	$\pm\sigma$.	$A \pm E_A$.	Difference between b and a in Averages.		$v \pm E_v$.	Difference between b and a in Coëfficients of Variability.	
						Diff. $\pm E_{Diff.}$	Diff./$E_{Diff.}$		Diff. $\pm E_{Diff.}$	Diff./$E_{Diff.}$
Males	1	(*a*) "Thyroid"	57	1.37	17.18 ± 0.12	+0.15 ± 0.17	0.9	7.97 ± 0.50	−1.10 ± 0.71	1.5
		(*b*) Control.....	42	1.19	17.33 ± 0.12			6.87 ± 0.51		
	2	(*a*) "Thyroid"	65	1.21	17.15 ± 0.08	+0.10 ± 0.11	0.9	7.06 ± 0.42	−1.96 ± 0.53	3.7
		(*b*) Control.....	57	0.88	17.25 ± 0.08			5.10 ± 0.32		
	3	(*a*) "Thyroid"	46	1.12	18.72 ± 0.11	+0.28 ± 0.19	1.5	5.98 ± 0.42	+1.55 ± 0.74	2.1
		(*b*) Control.....	35	1.43	19.00 ± 0.16			7.53 ± 0.61		
	4	(*a*) "Thyroid"	50	1.18	16.58 ± 0.11	+0.01 ± 0.19	0.05	7.12 ± 0.48	−0.31 ± 0.78	0.4
		(*b*) Control.....	27	1.13	16.59 ± 0.15			6.81 ± 0.62		
Females	1	(*a*) "Thyroid"	53	1.54	15.87 ± 0.14	+0.16 ± 0.18	0.9	9.70 ± 0.64	−0.65 ± 0.84	0.8
		(*b*) Control.....	63	1.45	16.03 ± 0.12			9.05 ± 0.54		
	2	(*a*) "Thyroid"	47	1.17	15.89 ± 0.12	−0.09 ± 0.17	0.5	7.36 ± 0.51	+1.63 ± 0.76	2.1
		(*b*) Control.....	59	1.42	15.80 ± 0.12			8.99 ± 0.56		
	3	(*a*) "Thyroid"	34	1.31	16.65 ± 0.15	+0.45 ± 0.18	2.5	7.87 ± 0.64	−1.55 ± 0.77	2.0
		(*b*) Control.....	50	1.08	17.10 ± 0.10			6.32 ± 0.43		
	4	(*a*) "Thyroid .. "...	48	1.71	15.58 ± 0.17	−0.19 ± 0.23	0.8	10.98 ± 0.76	−0.91 ± 1.03	0.9
		(*b*) Control.....	49	1.55	15.39 ± 0.15			10.07 ± 0.69		

upon (cf., *e.g.*, the manual of Biedl, '22, as well as the papers of Romeis, '14–'15, '16 and '18, Gedroyć, '23, Deutsch, '23, and others). Consequently the absence of any considerable and constant differences in the processes of metamorphosis between my control animals and those fed on thyroid, does not solve our problem. We ought to take into consideration, that owing to individual fluctuations on the starting day of the thyroid feeding, *i.e.* on June 5th, the larvæ experimented upon without any doubt differed from one another considerably both in regard to weight and most probably also to stage of development, although having hatched during one and the same night. It therefore was a priori not known whether the average terms of metamorphosis remained unchanged in spite of the different influence exerted by thyroid on separate specimens. For instance, if the positive and the negative influence exerted by thyroid feeding on separate caterpillars were of identical intensity, the average duration of the larval as well as of the pupal stage would remain unchanged. The so to speak latent influence of thyroid feeding ought to be noticeable here only by the variation of the terms of metamorphosis, as the range of individual fluctuations of the terms of pupation and emergence of adult moths would in such case undergo in the experimental materials a distinct widening. As a matter of fact in my experiments no remarkable changes in the coefficients of variability of these terms of the mentioned variation may be seen (cf. Tables I. and II.). The differences of the appertaining coefficients between control and thyroid materials have opposite signs, in one case being positive, in the remaining negative. The ratio of the difference between the coefficients to its probable error was only in few cases larger than 3, as a rule attaining from 0.2 to 2.3 only. It follows therefrom that these biometrical tests show no distinct influence of thyroid feeding on the rate of metamorphosis of the insects experimented.

The above results require a certain supplement in still another direction. Viz: the question arises whether in these experiments the lack of influence of thyroid on metamorphosis is caused by too small quantities of the substance added, or unsuitable age of the animals at the beginning of the experiments. Decisive

answers to this question have been attained, according to my view, by weighing fresh pupæ. It appeared that the growth of the caterpillars (the term of which was determined by the weight of fresh chrysalids) underwent in all cases, without any exception, a considerable retardation under the influence of thyroid feeding (cf. Table III.). The differences between the control and the experimental material are here always positive, in 3 cases being biometrically very significant. The thyroid had therefore in this respect an undoubtedly negative influence on the processes of larval growth, *i.e.*, the dosing of thyroid as well as the age of the animals have been well chosen to demonstrate the supposed influence of this substance. It is very characteristic that the variability of weight of the "thyroid" caterpillars does not undergo any essential and corresponding changes (cf. Table III.). This points to the fact that the negative influence exerted here by thyroid feeding on growth of caterpillars had the same qualitative and quantitative effect for all animals.

The influence of organic or even inorganic compounds of iodine on amphibian metamorphosis is much discussed in recent years and the problem contains certain discrepancies (cf., *e.g.*, the papers of Romeis, '18, Allen, '19, Hirschler, '18–'19 and '22, Huxley, '22, Huxley and Hogben, '22, Uhlenhuth, '22, and others). Gedroyć ('23) studied the problem in respect to insects, viz. on *Deilephila euphorbiæ* L. In two experiments each containing 5 "iodine" caterpillars, the author observed an acceleration of the pupation of the experimental specimens relatively to the 15 control caterpillars. On the contrary, in the third analogous experiment in which also 5 animals, but 2 or 4 days younger than the larvæ of the preceding series, were used, the larval period underwent in the "iodine" material even a certain prolongation. Hence the inference of Gedroyć as to the decisive influence of age and "state of growth" of the animals exerted on the direction of the action of iodine.

The material of Gedroyć being scanty I have studied anew the problem of the influence of iodine on insect metamorphosis. My experiments were performed on caterpillars of *Pieris brassicæ* L. Larvæ hatched between August 2d and 3d were used in all 4 experiments. Cabbage leaves abundantly sprayed with Lugol's

TABLE III.

DATA CONCERNING THE WEIGHT OF CHRYSALIDS OF *Lymantria dispar* L., IN MG.

n, number of specimens; σ, standard deviation; $A \pm E_A$, average weight together with its probable error; $v \pm E_v$, coëfficient of variability of the weight together with its probable error; Diff. $\pm E_{Diff.}$, difference together with its probable error; Diff./$E_{Diff.}$, ratio of the difference to its probable error.

Sex.	Number Corresponding to Each Experiment.	Material of Chrysalids.	*n*.	$\pm\sigma$.	$A \pm E_A$.	Difference between *b* and *a* in Averages.		$v \pm E_v$.	Difference between *b* and *a* in Coëfficients of Variability.	
						Diff. $\pm E_{Diff.}$	Diff./$E_{Diff.}$		Diff. $\pm E_{Diff.}$	Diff./$E_{Diff.}$
Males	1	(*a*) "Thyroid"	59	70.6	367.0± 6.2	+14.1±10.7	1.3	19.2±1.24	+3.6±2.10	1.7
		(*b*) Control.....	45	86.8	381.1± 8.7			22.8±1.70		
	2	(*a*) "Thyroid"	68	69.5	408.8± 5.7	+18.8± 9.4	2.0	17.0±1.01	+2.7±1.63	1.7
		(*b*) Control.....	58	84.3	427.6± 7.5			19.7±1.28		
	3	(*a*) "Thyroid"	50	89.9	428.0± 8.6	+11.7±12.4	0.9	21.0±1.48	−2.3±2.09	1.1
		(*b*) Control.....	39	82.2	439.7± 8.9			18.7±1.48		
	4	(*a*) "Thyroid"	57	79.6	430.7± 7.1	+60.7±13.5	4.5	18.5±1.21	+0.2±2.09	0.1
		(*b*) Control.....	29	92.1	491.4±11.5			18.7±1.71		
Females	1	(*a*) "Thyroid"	55	248.4	1078.2±22.6	+90.6±32.5	2.8	23.0±1.56	+0.7±2.16	0.3
		(*b*) Control.....	64	277.4	1168.8±23.4			23.7±1.49		
	2	(*a*) "Thyroid"	48	299.6	1383.4±29.2	+92.8±42.4	2.2	21.7±1.56	+2.0±2.20	0.9
		(*b*) Control.....	59	350.5	1476.2±30.8			23.7±1.55		
	3	(*a*) "Thyroid"	37	307.8	1175.6±34.1	+216.6±46.6	4.6	26.8±2.25	−2.4±2.83	0.8
		(*b*) Control.....	52	340.3	1392.2±31.8			24.4±1.71		
	4	(*a*) "Thyroid"	48	237.7	1187.6±23.1	+440.4±41.4	10.6	20.0±1.43	+2.1±2.12	1.0
		(*b*) Control.....	50	359.3	1628.0±34.3			22.1±1.56		

solution (6 g. IK, 4 g. I in 100 g. H_2O) were daily administered to the caterpillars of experiments 1 and 2 from August 16th, and in those of experiments 3 and 4 from August 22d. Leaves designed for control animals were similarly changed daily and sprayed with water. All animals were kept under glass vessels. The administered leaves were taken by the "iodine" caterpillars as well as by those of control materials. Data referring to the pupation *term* of these caterpillars as well as to the average *weight* of the resulting chrysalids are recorded in Table IV.

TABLE IV.

DATA CONCERNING THE TERM OF PUPATION AND THE WEIGHT OF PUPÆ FROM "IODINE" CATERPILLARS OF *Pieris brassicæ* L.

The terms of pupation calculated from the beginning of iodine feeding.

Number Corresponding to Each Experiment.	Material of Specimens.	Number of Specimens.	The Average Term of Pupation, in Days.	Difference between *b* and *a*.	The Average Weight of Chrysalids, in mg.	Difference between *b* and *a*.
1	(*a*) "Iodine" ..	12	12.2	− 2.4	294.4	+ 76.5
	(*b*) Control....	23	9.8		370.9	
2	(*a*) "Iodine" ..	11	12.1	+ 0.9	364.1	+ 10.9
	(*b*) Control....	15	13.0		375.0	
3	(*a*) "Iodine" ..	8	8.5	+ 2.5	311.5	+ 67.1
	(*b*) Control....	10	11.0		378.6	
4	(*a*) "Iodine" ..	6	12.0	− 2.7	251.7	+ 59.6
	(*b*) Control....	6	9.3		311.3	

It is evident that neither in the case when iodine feeding was begun earlier (first and second experiment) nor in that started almost a week later (third and fourth experiment) the obtained results were concordant. The sign of the difference between the terms in control animals and those for the experimental materials was different in both cases which points to the lack of any influence of iodine feeding. On the other hand the average weight of the "iodine" chrysalids was here also always smaller than that of the control pupæ. The total of the recorded chrysalids amounted here to 37 "iodine" and 54 control specimens. My material was therefore larger than that of Gedroyć. It was nevertheless not large enough to consider the results obtained as decisive, all the more as the insects were not

segregated in respect to sex. Further and more detailed research ought to be undertaken in this respect.

It ought to be remarked, that notwithstanding such distinct influence of both thyroid and Lugols' solution on growth of caterpillars, mortality in the animals of the experimental materials was in general not larger than in those of the control. The average mortality in male "thyroid" larvæ attains ca. 6 per cent., in females 5 per cent., in the control materials 4 per cent. in males and 7 per cent. in females. Analogous data for "thyroid" chrysalids was 7 and 3 per cent., in control pupæ 6 and 2 per cent. The larvæ of *Pieris brassicæ* reared as a rule very badly in the extremely damp air under glass, but also here no specially negative influence of the experiment could be observed. Viz. the average mortality in the "iodine" caterpillars was here approximately 43 per cent., in the control material 38 per cent.

TABLE V.

THE COËFFICIENTS OF CORRELATION BETWEEN THE WEIGHT OF CHRYSALIDS OF *Lymantria dispar* L. AND THE LENGTH OF THE LARVAL PERIOD.

$r \pm E_r$, coëfficient of correlation together with its probable error; r/E_r, ratio of the coëfficient to its probable error.

Material of Specimens.	Number Corresponding to Each Experiment.	Males.		Females.	
		$r \pm E_r$.	r/E_r.	$r \pm E_r$.	r/E_r.
"Thyroid"	1	− .425 ± .072	5.9	+ .095 ± .090	1.1
	2	− .161 ± .080	2.0	− .408 ± .081	5.0
	3	− .506 ± .071	7.1	− .207 ± .106	2.0
	4	− .652 ± .051	12.8	− .251 ± .091	2.8
Control.	1	− .476 ± .078	6.1	+ .033 ± .084	0.4
	2	− .278 ± .082	3.4	+ .460 ± .069	6.7
	3	− .415 ± .089	4.7	− .130 ± .092	1.4
	4	− .606 ± .079	7.7	+ .279 ± .088	3.2

After the present experiments were finished an abstract appeared [1] referring to a recently published preliminary note of Terao and Wakamori ('24) inaccessible to me. The authors report that the metamorphosis of the caterpillars of *Bombyx*

[1] *Berichte ü. d. ges. Pysiol. u. exp. Pharmak.*, Vol. 28, p. 119, November 12, 1924.

mori L. fed on mulberry leaves sprayed with thyroid extract is normal and that the "thyroid" animals are smaller than the controls. The accelerating influence of the thyroid food was however noticed in the second generation of the insects under examination. We see that the results of my "thyroid" experiments are in complete agreement with those performed by Terao and Wakamori on the first generation of the animals. Unfortunately I have been unable to examine the second generation of my moths.

I will not discuss here the relation of the lack of any influence of thyroid on the rate of metamorphosis of the insects experimented upon to the problem of the physiology of metamorphosis in these animals. As I have emphasized elsewhere (Kopeć, '23), only positive results of the experiments on the influence of glands of internal secretion may be considered as decisive in this respect and only to a certain degree. It is not impossible that the negative influence exerted in my experiments on the weight of caterpillars by thyroid feeding is not due to specific checking of growth faculties, but rather to certain changes elicited in their digestion by a totally foreign substance. Such caution is the more advisable, as we have already certain data in the literature, which point to positive effect exerted on "lower" animals by substances of glands of internal secretion from vertebrates. Nowikoff ('08) remarks an increase of the rate of multiplication of infusorians reared in aqueous extracts of the thyroid gland. Hankó ('12) observes a positive influence of extracts of hypophysis on the rate of moult, regeneration and growth in *Asellus aquaticus*. The thyroid gland has, according to Wulzen ('16) a positive influence on the rate of division and growth of *Planaria maculata*. From the experiments of van Herwerden ('23) we see that the cortical substance of adrenals of the ox has an accelerating influence on the multiplication of *Daphnia pulex* as well as on growth of this crustacean and of young specimens of *Limnæa stagnalis*. I believe however that only a larger experimental material may constitute a base for general discussion on the influence of glands of internal secretion of vertebrates on processes taking place in invertebrates.

The opinions of separate authors as to the relation of growth

to metamorphosis are not as yet in agreement. While for certain authors metamorphosis is a function of growth—for others there is no essential connection between the two processes. My experiments furnish a new support for the latter opinion, if of course we shall agree to draw inferences as to growth from the weight of the animals. We see that in "thyroid" caterpillars notwithstanding a considerably decreased weight, the rate of metamorphosis remains totally unchanged. If metamorphosis were a function of growth, the correlation between the weight of the pupæ and the duration of larval life would be, at least in normal conditions, always negative. It is therefore very characteristic that both in the "thyroid" and in the normal material the coefficients of correlation between the weight of the pupæ (*i.e.*, the measure of growth) and the duration of the larval period were, it is true, in general rather large, but not always negative (cf. Table V.). According to my opinion we may infer therefrom that between larger growth and quicker metamorphosis of caterpillars there is only the relation of contemporariness, perhaps dependent on a totally separate, unknown factor (or factors). We have not to do here with real, functional correlation between growth and metamorphosis which could not be abolished even in normal conditions.

In conclusion I want to remark that the results of my present research are by no means contradictory to my former views on the decisive rôle of the brain for insect metamorphosis, during normal development (Kopeć, '22) as well as during starvation (Kopeć, '24). The substance of the thyroid gland from vertebrates may have no relation to the substance (or substances) the existence of which ought, according to my opinion, to be considered as indispensable for the metamorphosis of these animals. I refer their presence in the insect to the presence of brain. The lack of metamorphosis in the caterpillars of *Lymantria dispar* which have been deprived of brain the seventh day after their last moult (Kopeć, '22) is sufficient evidence in this connection. The only matter for discussion is whether this influence occurs through the interaction of nerves, or, according to my supposition (not assertion), by means of internal secretion. In recent times Gedroyć ('23) opposed very decisively my

interpretation of the hormonal rôle of the brain during insect metamorphosis. Namely when grafting brains or other parts of the nervous system on normal caterpillars of *Deilephila euphorbiæ* this author did not observe any acceleration of metamorphosis. I cannot, however, consider as decisive experiments like those of Gedroyć, in which the influence of grafted parts of the nervous system are studied, the analogical parts of the host not being removed. The introduced nervous tissue is present here in excess and it *might* at most, but it *need* not elicit accelerated metamorphosis, *i.e.*, increase the effect of the function which brain probably has when present in the organism in normal quantities.

I want to explain here a certain seeming contradiction between the results of my former experiments, which has been justly pointed to by the mentioned author. Gedroyć emphasizes that from Table I. of my paper on the rôle of the brain in the metamorphosis of insects (cf. Kopeć, '22) it follows that the control caterpillars which fasted since the seventh day after their last moult, and the head of which has been injured, the brain being not removed undergo pupation simultaneously with the caterpillars which have not been operated upon at all, and which were not deprived of food. On the contrary from my later paper on the influence of starvation on the development of insects (cf. Kopeć, '24) it follows that caterpillars which have been subjected to inanition since the seventh day after their last moult undergo pupation later than the unstarved controls. This discrepancy is however not essential for the caterpillars subjected in either case to starvation the seventh day after their last moult were not physiologically identical. In the second case (Kopeć, '24) they were normally fasting animals, in the first (Kopeć, '22) injured similarly to those the brain of which has been removed. Owing to the severe injury, connected with loss of blood, these caterpillars were not able to take food and were therefore subjected indirectly to starvation. I have twice just emphasized in connection with other experiments (Kopeć, '08 and '11) that serious operations on caterpillars may have an accelerating influence on the succeeding moult, which in the above mentioned case corresponded to pupation. This acceler-

ating influence on metamorphosis may thus have annulled in the case from my paper of 1922 the hindering influence of starvation. In other words, probably owing to mutual counteraction of the two opposite influences the normal term of pupation was maintained here.

From the foregoing inquiry the following summary may be given:

1. The administration of thyroid to caterpillars of *Lymantria dispar* L. did not cause any distinct changes in the duration of the larval nor of the pupal period. The only effect was a distinct diminution of the weight of the chrysalids.

2. Neither the variability of the pupation term, nor that of the emergence of the moths, nor of the pupal weight underwent any regular or essential changes as compared with corresponding items of the control materials.

3. Lugols' solution (IK + I) had no distinct influence on the rate of pupation of the caterpillars of *Pieris brassicæ* L. and its only consequence was the negative changes of pupal weight. In respect to the scanty material these experiments are however not considered as decisive.

4. Negative correlation between the growth of caterpillars of *Lymantria dispar* L. (the growth being expressed as weight of the pupæ) and the duration of larval life cannot always be observed. This fact as well as the diminution of growth in spite of the unaltered rate of metamorphosis in the "thyroid" experiments point to phenomena of concommitance but not of functional correlation normally existing between metamorphosis and growth of insects.

5. The mortality of the "thyroid" as well as of the "iodine" specimens was not larger than in the control materials.

6. The hypothesis formerly uttered by the author as to the hormonal influence exerted by the brain on insect metamorphosis is by no means abolished by the negative results of the present experiments. Neither is this hypothesis cancelled by the experiments and considerations of Gedroyć.

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SINGLE AND DOUBLE RINGS AT THE REDUCTION DIVISION IN UVULARIA.

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It has not, apparently, been yet ascertained in what points the reduction division in flowering plants differs from the corresponding division in animals, such as the insects (see Tischler, 1922). The absence of a centrosome, indeed, has been noted in many flowering plants; and it has also been presumed that the division of the homologous chromosomes into chromatids is not visible before the early anaphase. But whether the complicated changes described, for instance, by Janssens (1924) as occurring in insect chromosomes and summarized by Wilson (1925), are paralleled in flowering plants, is, it appears, not known (though Chodat, 1925, considers that *Allium* offers a parallel). The following is a small contribution to the determination of the likenesses and differences of the maturation divisions of flowering plants and those of the best known animals, say, the Orthoptera.

As compared with most animals, flowering plants may differ in showing a periodicity in the occurrence of the maturation divisions corresponding with the alternations of day and night. Certain stages of *Uvularia*, for example, apparently usually came late at night and hence were rarely obtained under normal environment in the daytime. However, cold checks or stops the process; and if preparations are made at intervals after cold has occured in the night, various less common stages can often be procured in the daytime. The first and second metaphases of *Uvularia* have been readily obtainable under the usual environment; but the stages just previous to the first metaphase, and those showing the separation of the bivalents into their component chromosomes, or chromatids, were not often seen when preparations were made at 9 or 10 o'clock in the morning. The preparations were mostly procured after the young buds had been somewhat chilled by the temporary lowering of the night

temperature in February and March, when the greenhouse fires were banked.

The preparations of young microspores and pollen-mother-cells were fixed and stained in iron-acetocarmine (Belling, 1921). A water-immersion objective was used to examine the chromosomes. It may be noted that an apochromatic water-immersion of 1.25 aperture will allow a true condenser aperture of 1.2, if the source of light is diaphragmed to fit the field of view. The working aperture of the combination is about 1.2. On the other hand, the working aperture of the 1.4 N.A. apochromatic oil-immersion, used with a dry Abbe uncorrected condenser on objects in iron-acetocarmine, is only 0.9 or less. (Compare Belling, 1923.)

Three hypotheses have been made as to the rings seen at the metaphase in certain Liliaceæ and other genera of flowering plants. (1) That these rings result from the mere twisting of the constituent chromosomes of the bivalents around one another, and that these become untwisted and longitudinally split at the anaphase. This hypothesis is, it would seem, negatived by the fact that, with the best visibility in the microscope, it is usually impossible to say which chromosome is above and which below at the junctions. (2) That the rings are caused by alternate openings between the homologues and between their constituent chromatids, so that adjacent rings are always at right angles. This hypothesis does not appear to agree well with the state of affairs in the trivalents of the triploid hyacinths, where 3 chromosomes are concerned (Belling, 1925*a*). (3) That between each two adjacent rings there is a chiasma in Janssens' sense (or a similar interlacing of strands) where segmental exchange may have taken place, by the fracture and subsequent reciprocal junction of two of the four strands. This assumption seems most suitable for a working hypothesis, since crossing-over (and hence probably segmental interchange) has been shown to occur in those flowering plants which have been investigated *ad hoc*.

However, in the present paper only the following points are regarded.

1. Whether in horizontal rings or V's one chromatid passes up and one down from each lateral half of the ring or V.

2. Whether these chromatids in separating show signs of inter-

lacing at the junction, so that a ring or V gradually diminishes in size as its chromatids are pulled into the loop by the spindle fibers.

3. Whether, in vertical rings or V's, the process is the same as the above; or whether the upper and lower halves of the rings or V's separate as wholes.

4. What different configurations are shown by the same homologous chromosomes in different cells.

Bivalent I.

Chromosome I., in the pollen grain (Fig. 1), is seen to be formed of two slightly unequal segments, and is usually bent; the spindle

Fig. 1. This shows the metaphase at the first division in the pollen-grain. (Like all the other figures it was made from preparations of *Uvularia grandiflora*.) This pollen-grain was selected from a large number as showing the chromosomes well spaced. The longitudinal divisions and the constrictions are evident. It can be seen that the two segments of chromosome I. are of unequal length. Chromosome III. has apparently a shorter long segment and a longer short segment than chromosome II. The small segment of chromosome V. is closely attached to the large segment, while in chromosome VI., the two segments are separated by a thread.

This, and the subsequent figures were drawn with the camera from iron-aceto-carmine preparations, the light being screened by Wratten yellow-green filter No. 56. A Leitz' achromatic aplanatic condenser, corrected for use as a water-immersion, was used to give a large cone of light. Zeiss' apochromatic water-immersion 70 was employed in all cases.

fiber being, of course, attached at the constriction, where the two segments are connected by a fine thread. Fig. 2 of the paper on the origin of chromosomal mutations (Belling, 1925*b*) shows bivalent I. in the late prophase in the form of two medium-sized

rings with a long V, the plane of the V being at right angles to that of the adjacent ring. At the metaphase, as shown in Fig. 2 of the present paper, one large ring and V may be present. The

FIG. 2. First metaphase (to anaphase) in a pollen-mother-cell, from a plant forced in the greenhouse, in March, 1923. This cell was selected as showing no overlapping bivalents. In bivalents I., II. and III., the spindle fibers would probably be attached where the constituent chromosomes seem to cross.

division into chromatids cannot be seen in face view, but only when the rings or V's are presented edgeways. In the next stage, which is not figured here, a small loop arises from the

FIG. 3. First metaphase to anaphase in a pollen-mother-cell. Drawn in March, 1924; after a spell of cold weather. The drawing surface was shifted after outlining each bivalent, as was done also in Figs. 4 and 5. Bivalent VI. has the more or less exceptional configuration noted in the text in bivalent IV. Bivalent VII. is the most advanced in separation.

apposed points of constriction of the homologues, and is pulled out by the spindle fibers towards each pole, pulling the chromatids out of the rings or V's as it apparently increases in size at the expense of these rings or V's. In Fig. 3, bivalent I. consists of a large loop with two medium-sized rings; one of which is seen edgeways. This ring shows the division into chromatids. This bivalent perhaps started as two rings. In Fig. 5, lower line,

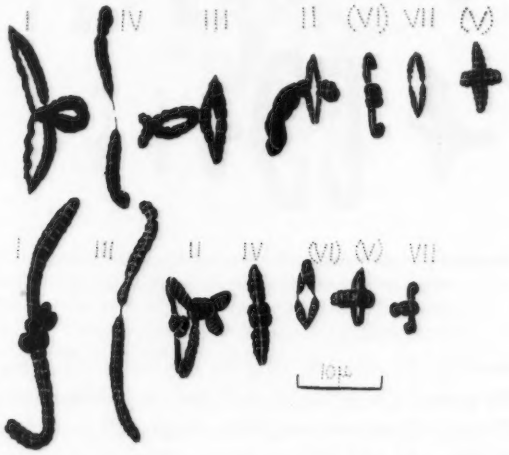

FIG. 4. Bivalents from two more selected cells showing the metaphase to anaphase, as in Fig. 3. Two bivalents, VI. and VII., are of the exceptional form.

bivalent I. consists of a diminishing ring and a V, with a large loop between them, formed probably at their expense. Bivalent I., in the upper line of this figure, is apparently similar but the ring is somewhat bent. In Fig. 3, of the paper on the origin of mutations, bivalent I. has the form of a large loop with two small rings, probably remnants of original large rings. In Fig. 4 of the present paper, upper line, bivalent I. has a ring on one side of the loop, while on the other side the ring may perhaps have been vertical, and the two halves have separated vertically. In the lower line of this figure, the halves of bivalent I. have separated at one end, but the last remnant of the V remains at the center. Finally, in Fig. 6, the 4 chromatids of bivalent I. are

clearly visible; and it may be that there had been a horizontal ring on the right, and a vertical ring on the left.

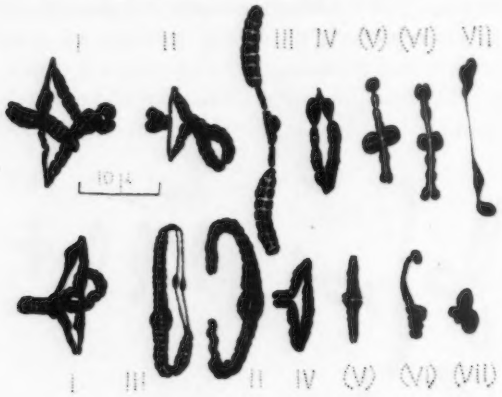

FIG. 5. Two more selected metaphase to anaphase groups. The last two bivalents in the lower line seem more or less malformed.

BIVALENTS II AND III.

Chromosomes II. and III. (Fig. 1) doubtless differ slightly in size, but are similar in behavior, and may be considered together. They are long J chromosomes, with a large and a small segment close together. At the late prophase (Fig. 2, Belling, 1925*b*) these two bivalents may have each the form of a large horizontal ring with a small V at one end, and a vertical portion consisting of the two small segments at the other end. At the metaphase (in Fig. 2, of the present paper), one of these bivalents forms a ring with a small V, and the other a large and small ring in different planes. Fig. 4 of the paper on the origin of mutations shows bivalent II. forming a horizontal ring with a small vertical piece consisting of the two small segments, and with a small loop arising from the constrictions. In the upper line of Fig. 4 of the present paper, this loop has increased in size in bivalent II. In Fig. 4, lower line, and Fig. 5, upper line, bivalent II. shows part of the small segments still in the equatorial plane, as well as a ring (or ring and V) on the other side. But in bivalents II. and III. of Fig. 3, the small segments have been quite drawn into the loop, and only the diminishing V is left; or in bivalent III. of the

upper line of Fig. 4, the ring and V. In Fig. 5, lower line, both short and long segments have been drawn into the loop. Fig. 6 is especially instructive, for bivalent II. probably had consisted of

FIG. 6. A rare stage of the pollen-mother-cell at the early anaphase, showing the final separation of bivalents I., II. and III. The arrangement of the chromatids of bivalent II. must apparently be due to the horizontal splitting of a horizontal ring. The four smaller bivalents are, as usual, ahead of the larger ones in completing the separation of their constituents.

a large horizontal ring with a vertical piece formed of the two short segments. This is seen to have separated by the horizontal splitting of the ring into its constituent chromatids, the small segments having first separated. In bivalent III. of Fig. 6, a horizontal V has, it seems, separated into chromatids in the same way.

BIVALENT IV.

Chromosome IV., as shown in Fig. 1, consists of a small J, in which the two segments are close together. It is distinctly larger than either V. or VI. In Fig. 2 of the paper on the origin of mutations, bivalent IV. is formed of a horizontal V, with a vertical piece consisting of the two short segments. In Fig. 2 of the present paper, this bivalent, which apparently started as a ring, is already in the early anaphase (for the small bivalents usually separate before the large ones). In Fig. 3, bivalent IV., which possibly started as a ring and V, still shows a small V at the left of the loop. A later stage is shown in the lower line of Fig. 5. In the top of Fig. 4, the two halves of bivalent IV. have separated

without splitting lengthways. The constriction between the short and long segments is visible. The upper line of Fig. 5 shows a different configuration for bivalent IV., in which the two sides of the loop are equal. This is intelligible, first if we suppose the point of attachment of the spindle fiber and the constriction to have been shifted to the center of both chromosomes, which seems unlikely. Or we may presume that a horizontal V has separated into its chromatids, somewhat as bivalent III. of Fig. 6.

Bivalents V. and VI.

Chromosomes V. and VI., since they differ only in VI. having its small segment attached by a long filament, cannot be told apart in the maturation divisions, and must therefore, be considered together. In Fig. 2 of the paper on the origin of mutations, bivalents V. and VI. have the form of V's or truncated A's. In Fig. 3 of the present paper, in the configuration marked (V.), the two small segments have separated, and the two large segments are still horizontal and show the split between the chromatids. Similar forms may be seen in the top line of Fig. 5. The bivalent marked (VI.) in Fig. 3, however, like the one in the bottom line of Fig. 4, is presumed to have come from the separation of the chromatids of a horizontal V.

Bivalent VII.

Finally bivalent VII. resembles V. and VI. in its configurations, but is recognizably smaller.

To sum up: (1) There is, apparently, fusion at the junctions of the two constituents of each bivalent. (2) Horizontal rings and V's divide into their constituent chromatids along a horizontal plane, the line of division being distinct at the metaphase. (3) Vertical V's and rings have not been observed in the act of separating; but they probably separate into upper and lower halves. (4) The rings and V's apparently diminish as the loop between them is pulled out by the spindle fibers. This would presuppose crossing or interlacing of the chromatids.

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